2020

Evaluating the Effects of Yeast Cell Wall Component, Phytochemical Oil, and Vitamin E on Sow Antioxidant Status and Growth Performance of Dam and Offspring

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EVALUATING THE EFFECTS OF YEAST CELL WALL COMPONENT, PHYTOCHEMICAL OIL, AND VITAMIN E ON SOW ANTIOXIDANT STATUS AND GROWTH PERFORMANCE OF DAM AND OFFSPRING

BY

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A thesis submitted in partial fulfillment of the requirements for the Master of Science

Major in Animal Science

South Dakota State University

2020

THESIS ACCEPTANCE PAGE

Lily P. Hernandez

This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ACKNOWLEDGMENTS

I will forever be thankful to my advisor, Dr. Crystal Levesque, for providing me this opportunity. During my time under your mentorship you never failed to push me when I needed it, encouraged me through my struggles, but most importantly, you taught me how to keep improving. I would like to send my appreciation to Dr. Ryan Samuel for his participation as a committee member and support with this thesis. I would also like to send my thanks to both Dr. Benoit St. Pierre and Emily Fowler for their assistance with the microbial analysis of my samples.

Thank you to the Foundation for Food and Agriculture Research and ADM Animal Nutrition for funding this project. Thank you once more to ADM Animal Nutrition for the provision of all test ingredients used in the studies. To Jim L. Dunn and Joel Wenninghoff from ADM, thank you for the constant support you provided throughout my time at South Dakota State University. Your encouragement was invaluable to my progress and is something I will forever be grateful for.

I am forever thankful to the past and current members of Dr. Levesque's lab group for their assistance in data collection, especially Jamie Ortman, Ron Navales, Katelyn Zeamer, Talia Everding, Christian Ramirez, Tiffany Bruhn, Dr. Jodi Morton, and Dr. Jorge Yair Perez Palencia. I would also like to voice my appreciation to Shannon Dierking, Rhuan Filipe, Maíra Resende, and Analicia Swanson for their friendship during my last year in the program. As well, thank you to the other graduate students in the department for their companionship during my two and a half years at SDSU. I would also like to acknowledge all of the faculty and staff members of the department that

helped guide and support me during my time here. Thank you all for making SDSU a second home for this Texan, I will truly miss you all.

To the SNaP team, Sarah Sprayberry, Logan Joiner, and Kara Dunmire, thank you three for taking me under your wing during my undergraduate years at Texas A&M. Thank you for believing in me and supporting me when I wanted to continue on and obtain my master's. I am forever grateful that our paths crossed during those years. To Rachel Park, thank you for the ever-flowing encouragement and support you presented me during my time here. Your overflowing optimism and words of reassurance has meant a lot to me.

I would like to thank my friends, Hazel Olsen, Marita Luo, and Harneet Sakaria, for their outpouring support during my time at SDSU. Thank you for always lending an ear to my ramblings, even if you were unable to fully understand the full extent of my research. Although we all are going down different paths in life, I have no doubt that we will always be in each other's lives.

Lastly, I would like to thank my family for the tremendous amount of support that they provided me during my time here at SDSU. To my siblings, Denise, Gigit, and Fernando, thank you for always being there when I needed someone to talk to or simply needed a distraction from the worries about my research. To my parents, Ada and Juventino Hernandez, gracias por los sacrificios que hicieron para darles a sus hijos la mejor vida que podrían tener. Te quiero muchísimo.

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ABBREVIATIONS

ADFI	Average daily feed intake
ADG	Average daily gain
AT	α-tocopherol
ATP	Adenosine-5-triphosphate
BF	Backfat
BW	Body weight
CAT	Catalase
CON	Control
d	day/days
EO	Essential Oil
ETC	Electron transport chain
g	Grams
G:F	Grain to feed ratio
GPX	Glutathione peroxidase
GSH	Glutathione

GSSG	Oxidized form of glutathione
GT	δ-tocopherol
h	hour
HNE	4-hydroxynonenal
i.m.	Intramuscular
IgA	Immunoglobulin a
IGF	Insulin-like growth factor
IGF-1	Insulin-like growth factor-1
IGF-2	Insulin-like growth factor-2
IGFBP	Insulin-like growth factor binding proteins
IgG	Immunoglobulin g
IUGR	Intra-uterine growth restriction
Kg	Kilogram
m	Meter
MDA	Malondialdehyde
ME	Metabolizable energy

MEI	Metabolizable energy intake
mm	Millimeter
MO	Mint oil
n	Number
PFA	Phytogenic feed additives
ppm	Parts per million
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SAS	Statistical analysis system
SOD	Superoxide dismutase
SR-BI	Scavenger receptor class B type I
YCMO	Yeast cell + mint oil

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ABSTRACT

EVALUATING THE EFFECTS OF A YEAST CELL WALL COMPONENT, PHYTOCHEMICAL OIL, AND VITAMIN E ON SOW ANTIOXIDANT STATUS, AND GROWTH PERFORMANCE IN DAM AND OFFSPRING

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Times of high metabolic activity in gestation and lactation, as well as periods of stress at weaning, can lead to greater incidences of oxidative stress in the dam and offspring during the suckling and post-weaning period. Oxidative stress is an imbalance between prooxidant molecules and the antioxidant defense system that can negatively impact growth and/or reproductive performance. The objective of this research was to evaluate the effectiveness of a yeast cell wall component (Citristim, ADM Nutrition, Quincy, IL), peppermint oil, and ɣ-tocopherol in gestation and lactation diets to alleviate the impact of oxidative stress on maternal reproductive and offspring growth performance from gestation to market. A total of 98 primiparous and multiparous females and their 1,086 offspring were used. In study 1, 45 sows and gilts (240.7 ± 38.5 kg BW) were assigned to one of 4 diets [Control diet (CON), control + yeast cell at 0.2% (YC), control + mint oil top dress at 10 ppm (MO), and control + yeast cell and mint oil top dress (YCMO)] provided from d110 of gestation through to weaning. Weaned offspring were randomly allotted to pens balanced by weight and litter (n=15-17 pens/treatment) within maternal treatment and received the same dietary treatment as the sow during the suckling phase for 35 d post-wean in a 4-phase feeding regimen. In study 2, 53 primiparous and multiparous dams (206.21 ± 35.26 kg BW) were allotted to 4 diet

regimens [Control (CON), control + yeast cell top dress at 0.15% (YC), control + mint oil top dress at 10 ppm (MO), and control + ɣ-tocopherol top dress at 200 ppm (GT)] from d5 post-breeding through to weaning. At weaning, piglets were randomly allotted to pens (n=11-19 pens/treatment), balanced by weight and litter within maternal treatment and fed a common diet for 126 d post-wean in a 9-phase feeding regimen. After d29 post-wean in study 2, performance of only pigs deemed light (<5.10 kg) and heavy (>7.25 kg) at weaning were followed to d126. Maternal dietary treatment did not impact sow BW, feed intake, piglet birth weight, and litter size in either study. Sows provided the YCMO diet in study 1 tended to have greater BF ($P < 0.10$) than YC sows at trial start and maintained a greater BF through to weaning. Superoxide dismutase activity in sow sera, colostrum, and milk did not differ between diets in either study. In study 1, glutathione content in milk tended to be lower ($P < 0.10$) in MO than YCMO sows with CON and YC intermediate. However, glutathione content in colostrum and d4 and 14 milk samples did not differ by maternal treatment in study 2. At weaning, piglets from YC sows were heavier ($P < 0.05$) than CON animals in study 1, while in study 2 piglets from GT-fed sows tended to be heavier ($P < 0.10$) at weaning than YC piglets both due to differences in daily gain. In the post-wean period for study 1, overall daily gain was greater ($P < 0.05$) for CON-fed pigs than YCMO pigs with overall feed intake greater ($P < 0.05$) for YCMO than MO resulting in lower ($P < 0.05$) gain:feed in YCMO-fed pigs vs CON and MO. In study 2, lightweight pigs from CON sows tended to be lighter ($P < 0.10$) than pigs from all other treatment groups at weaning and d29 post-wean due to differences in daily gain. Lightweight MO and GT pigs were heavier at d42 ($P < 0.05$) than CON and YC pigs. At d70 post-wean, GT pigs tended to be heavier than CON pigs, with YC and

MO intermediate. Lightweight pigs from MO sows had greater gain ($P < 0.05$) during the finishing period than all other treatment groups, with GT pigs gaining less. There were no detectable differences in BW during the finishing phase among treatments in heavyweight pigs, however, CON pigs tended ($P < 0.10$) to gain the least. Based on weight category distribution at d6 post-wean a larger percentage of heavy weight YC-fed pigs remained heavy compared to CON-fed pigs in study 1. A greater percentage of lightweight pigs provided the MO diets post-wean were average at the end of the trial period for study 1, with a lesser percentage of MO pigs deemed lightweight at d29 post-wean and a larger percentage classified as average in study 2. Inclusion of either test ingredient in both gestation and lactation diets did not impact sow performance with marginal influence on antioxidant status. Exposure to YC and GT during the suckling phase resulted in heavier offspring. However, prenatal and postnatal exposure to MO potentially provided lasting benefits to light-weight pigs resulting in these animals becoming average wean post-wean up to market. Addition of phytochemical oils, particularly mint oil, in gestation and or lactation diet may be advantageous in improving offspring performance from birth to market.

CHAPTER 1

1.0 LITERATURE REVIEW

1.1 Litter size and its implications on the sow

Swine industry performance as a whole relies ultimately on the efficiency of the sow. In this regard, continuous genetic selection over the past decades has led to highly prolific sows and production of highly lean progeny (Kim et al., 2013). The modern sow is leaner and larger in body size due to improved genetics that has also impacted the number of pigs born compared to the sow used years ago. Genus PIC data published by Tokach et al. (2019) showed an increase of 4.5 pigs per litter over the past 13 years, meaning approximately 0.35 pig per year improvement. Thus, modern sows are farrowing more than 15 total piglets born and the average pigs weaned per sow per year appears destined to pass 30 more pigs over the next few years. Although all these genetic improvements are beneficial to producers and the industry as a whole, there are negative implications and new challenges that arise with this advancement, within them: higher incidences of low birth weight piglets, increased within-litter weight variability, and increased preweaning mortality (Foxcroft et al., 2006, Quesnel et al., 2008). In this context, the drive to increase productivity continues, but now focused on the quality of piglets produced, thinking in kilograms of pork per sow per year as a better indicator of productivity rather than number of pigs produced per sow per year (Kitt, 2020).

In relation to sows, selection for total number born has also caused changes in sow physiology and metabolism, probably most evident during late gestation and lactation period (Kim et al., 2013). During these periods, there is an increase in the nutritional requirements of the sows due to greater fetal growth, development of

mammary tissue and milk production. However, during the lactation period, an issue arises when the dam is unable to consume enough feed to match the rate of milk production. Insufficient nutrient uptake leads to a catabolic state, which is how the organism responds to supply the nutrient deficiency and support milk production. Thus, during the catabolic state, sows use their body reserves to meet the demands of milk production for their offspring. A point can occur where body reserves have been exhausted, resulting in reduced milk yield and subsequent reproductive problems for the sow. In turn, the reduction in milk production would impair the performance of piglets during the suckling period, leading to lower gains and lighter wean weights. With lighter offspring at weaning, the need to ensure pigs reach market weight within the available time may result in increased labor and feed costs. Exhaustion of sow body reserves during the lactation period is not the only contributor to lessened sow productivity. During the period of body reserve mobilization, the female is experiencing high oxidative stress. Oxidative stress is linked to damaging both mitochondrial and cytoplasmic macromolecules (Roy et al., 2016) and lipid peroxidation. If no efforts are taken to alleviate this state, then serious damage is done to the body of the animal, which can create long-term effects on health and productivity of sows and offspring.

The addition of feed additives into sow diets may be an effective strategy in alleviating oxidative stress. Some research has looked at the inclusion of yeast-based products and antioxidant rich ingredients, such as phytochemical oils or vitamins (Tan et al., 2015; Meng et al., 2018; Lipiński et al., 2019; Reyes-Camacho et al., 2020). These studies suggest that inclusion of these types of products is successful in improving the oxidative status of the dam and alleviating the negative effects (Tan et al., 2015; Meng et

al., 2018; Lipiński et al., 2019; Reyes-Camacho et al., 2020). However, the results vary. Thus, there is a need for studies to determine if the use of these ingredients will actually alleviate the effects of oxidative stress and prevent reduced reproductive performance of the sow and the growth performance of their offspring as well as a better understanding of the mechanisms of how it happens.

1.2 Oxidative stress

Oxidative stress is defined as an imbalance between prooxidant molecules and the antioxidant system with the former produced in greater amounts that can potentially damage biological systems (Birben et al., 2012; Burton and Jauniaux, 2011; Ighodaro and Akinloye, 2018). Involvement of oxygen in physiological pathways highlights its importance in the cell. Most notably, oxygen participates in cellular respiration, the generation of adenosine-5-triphosphate (ATP) through oxidative phosphorylation (Burton and Jauniaux, 2011). The electronegative state of oxygen allows it to be a suitable electron acceptor. During transfer along the electron transport chain (ETC), electron leakage occurs frequently and results in the creation of free radicals. This is the outcome of when electrons exit early and react with oxygen forming superoxide ($O_2^{\bullet -}$) instead of continuing down the respiratory chain so the reduction of oxygen to water at cytochrome-c oxidase occurs (Jastroch et al., 2010). Free radicals encompass a vast array of molecules; most notably, they include reactive oxygen species (ROS) and reactive nitrogen species (RNS). The RNS play an integral part in cell damage, however, oxygen derivates are more important in comparison to nitrogen derivates in relation to diseases (Kurutas, 2016). The production of ROS is prominent in the cell, indicating that large concentrations are being made daily. However, only about 1–2% of the molecular oxygen

consumed during normal physiological respiration is converted into superoxide radicals (Ott et al., 2007).

Maintenance of ROS concentration by the antioxidant defense system occurred as a result of evolution. Primitive eukaryotic cells, sensitive to the presence of oxygen, created a defense system to protect the cell and its cellular macromolecules from damage (Ott et al., 2007). In a cell at rest, a homeostatic environment between ROS production and antioxidant activity is present. However, this balance is easily prone to disruption, indicating that even a slightly heightened production of ROS or deficient protective activity of antioxidants can cause the balance to sway. Figure 1-2 illustrates the fluctuation of ROS in the cell and its implication on the degree of oxidative stress that is present in the cell.

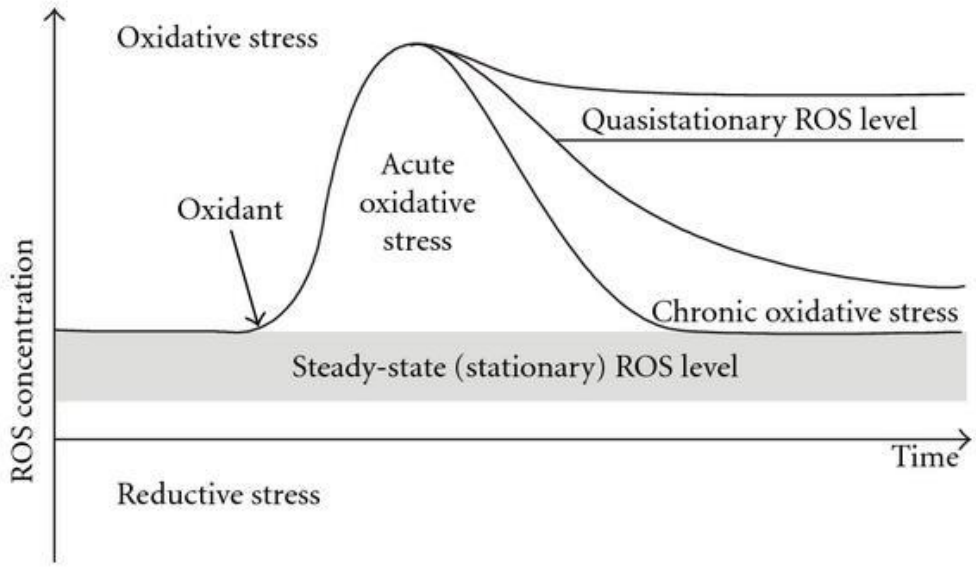

Figure 1.1. Dynamics of reactive oxygen species in biological systems [Adapted from Lushchak (2012)].

Normal ROS production fluctuates in the cell, although this fluctuation is unlikely to cause oxidative stress. Oxidative stress emerges from an enhanced ROS generation or

reduced antioxidant protective ability, being characterized by the reduced capacity of endogenous systems to fight against the oxidative attack directed towards target biomolecules (Pisoschi and Pop, 2015). There are different forms of oxidative stress that are dependent on the ability of the defense system to reduce ROS concentration. Acute oxidative stress, characterized by increased ROS production, is quickly reduced back to the initial (control) range of ROS (Lushchak, 2012). When the cell is kept in a state of increase ROS concentration for a longer period of time, potentially due to the defense system needing to create more antioxidants, that is identified as chronic oxidative stress.

Acute oxidative stress is less concerning as the length of time that a cell is under that state is minimal, whereas a cell under chronic oxidative stress has the potential to experience the damaging effects. When disturbance to the prooxidant-antioxidant balance is large enough, as observed with chronic oxidative stress, irreparable damage to organelles and molecules occur (Burton and Jauniaux, 2011). Interestingly, there are some circumstances where ROS levels do not return to the initial range and the system may be stabilized at new, higher ROS level referred to as “quasistationary”. There have been reports where the opposite situation of decreased ROS levels can occur and is sometimes called “reductive stress” (Lushchak, 2012). Reductive stress is defined as the emergence of excessive reducing equivalent, such as glutathione (GSH), in the presence of an intact oxidant-antioxidant balance (Lubos et al., 2011). Information surrounding reductive stress is continuously growing but Pérez-Torres et al. (2017) report that excess reducing equivalents diminish cell growth responses, induce alterations in the formation of disulfide bonds in proteins, reduce mitochondrial function and decrease cellular metabolism. Reports also state that chronic reductive stress can incite oxidative stress and

stimulate reductive stress by feedback regulation (Pérez-Torres et al., 2017). Thus, the varying degrees of oxidative stress that can occur, highlights the importance of the balance between ROS production and antioxidant activity.

Inability of the antioxidant defense system to reduce ROS to manageable levels and eliminate the state of oxidative stress results in the occurrence of oxidative damage. Oxidative damage results in modifications to molecules, such as DNA, most times leading to functions being compromised. If damage to a molecule is severe enough the repair would be unsuccessful; the organism is equipped with one final option, controlled cell suicide or apoptosis (Payne et al., 1995). Apoptosis allows the organism to halt further damage that could occur in neighboring organelles. Thus, inhibiting a systemic type of oxidative stress, occurs in several regions in the body. Most notably, free radical overproduction in the mitochondria is most dangerous as mitochondrial DNA is contained within the same organelle. An assessment of oxidative stress is necessary to return ROS production to stationary or controllable levels. Identifying markers of oxidative stress is necessary to pinpoint when a cell is under a state of stress.

1.3 Markers of oxidative stress

Excessive oxidative radicals are usually removed by a series of antioxidant molecules (Liu et al., 2017), which are classified into enzymatic and non-enzymatic. The reactions are believed to be performed in a step by step manner to establish a state of homeostasis in the cell (Figure 1-3). Superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT) are the three main enzymatic antioxidant molecules that have been related to oxidative stress in mammalian species (Shi-bin et al., 2007). These enzymes are a part of the first line of defense as they act to prevent the formation of free

radicals in the cell (Ighodaro and Akinloye, 2018). Along with these antioxidant molecules, there are several compounds that exhibit antioxidant activity and are considered to be part of the defense system. Differing opinions regarding classification of antioxidant molecules indicate that there are 2, 4, or 5 different levels in the defense system (Lykkesfeldt and Svendsen, 2007; Riachi and De Maria, 2015; Ighodaro and Akinloye, 2018).

Superoxide anion, hydrogen peroxide, and hydroxyl ion are the most notable types of ROS produced in the body. Review of the hydrogen peroxide structure illustrates that it does not possess free radicals, indicating that it should not be classified as a ROS (B; Figure1-3). However, its involvement in the generation and detoxification of free radicals allows for it to still fall under the ROS classification (Burton and Jauniaux, 2011). It is greatly important that the different levels of the antioxidant defense system work together. If the concentration of both superoxide and hydrogen peroxide is left neglected, the formation of hydroxyl ions, a more dangerous ROS with no known scavengers, occurs (Burton and Jauniaux, 2011). Thus, maintaining a homeostatic environment between ROS and antioxidants production within the body is important.

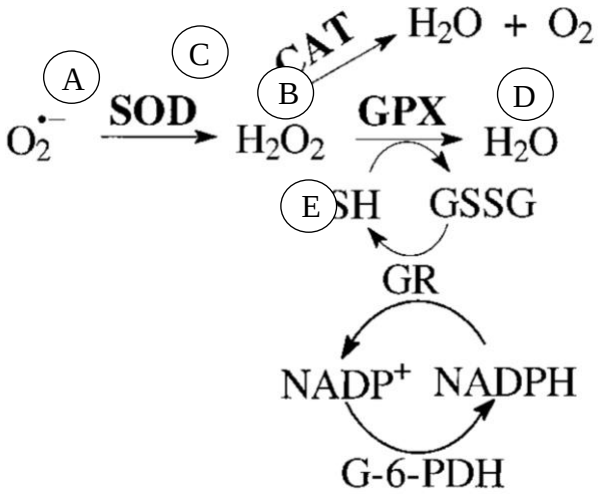

Figure 1.2. Antioxidant defense pathway. A- Superoxide anion, B- Hydrogen peroxide, C- Superoxide Dismutase enzyme, D- Glutathione Peroxidase enzyme, and E-reduced form of Glutathione. [adapted from Li et al. (2000)]

1.3.1 Superoxide Dismutase

The SOD molecule is considered to be an important antioxidant that acts as a major component of the first line of defense (Ighodaro and Akinloye, 2018). Once the presence of a superoxide ion is detected, SOD acts as a scavenger and seeks out the molecule. Dismutation of $O_2^{\bullet -}$ by SOD leads to the formation of hydrogen peroxide (Figure 1-3; Burton and Jauniaux, 2011). Hydrogen peroxide either remains in the immediate area to be further metabolized or diffuses across the membrane to the cytosol. Although the mitochondria electron transport chain is the most significant producer of mitochondrial ROS, mainly the superoxide anion (Fang et al., 2002; Ott et al., 2007), superoxide generation occurs in other regions of the cell. In order to accommodate for different production areas, different isoforms of SOD are present. In total, there are four different forms, Mn-SOD, Cu-SOD, Zn-SOD, and Fe-SOD (Ighodaro and Akinloye, 2018). In the mammalian species, only three of the four are found as Fe-SOD is solely located in plant cells (Gill and Tuteja, 2010; Karuppanpandian et al., 2011). Mn-SOD is located solely in the mitochondria, while both Cu and Zn-SOD are two isoforms that are located in the cytosol. As SOD is a metalloenzyme, requiring a metal ion cofactor for it to be active (Ighodaro and Akinloye, 2018), there is a need for a nutrient-rich diet. As SOD is the first antioxidant to fight against ROS in the cell, measuring SOD concentration in blood and other biological samples could potentially indicate if a cell is under a state of oxidative state. Konzack and Kietzmann (2014) indicated that the expression or activity

of the SOD molecules directly correlates with ROS levels. For example, the expression of antioxidant enzymes are lower in tumor tissues compared to healthy tissues. If high levels of ROS are found in the mitochondria or cytosol, then the amount of SOD present would decrease. In a state of chronic oxidative stress, the concentration of SOD generated by the defense system would be lowered as the amount of ROS produced exceeded the ability of the defense system to reduce is hindered.

1.3.2 Glutathione Peroxidase

Following the reduction of superoxide into hydrogen peroxide by SOD, hydrogen peroxide is successfully detoxified into water by CAT and GPX (Burton and Jauniaux, 2011). Similar to SOD, GPX can be located in both the mitochondria and the cytosol. Its presence in both regions requires several isoenzymes to ensure that the concentration of hydrogen peroxide is kept low. Four isoforms have been identified in mammalian cells, and of those four, three reduce hydrogen peroxide and peroxides of free fatty acids, whereas GPX-4 reduces peroxides of phospholipids and cholesterol (Toppo et al., 2009). Presence of multiple GPX enzymes is advantageous for the cell, however, the isoform of most importance is GPX-1.

Interestingly, GPX requires several secondary enzymes and cofactors to work efficiently (Li et al., 2000). As oppose to SOD, which solely requires a metal cofactor that could easily be obtained from the biological environment, GPX relies on cofactors which may or may not be present at the site of action. The most prominent cofactor involved in the activation of GPX is GSH. Both CAT and GPX work together with the GSH system. Glutathione is present in the cell either as free or bound to proteins (Zitka et al., 2012). Constant presence of this nonenzymatic protein indicates that GPX is readily

available for the detoxification of hydrogen peroxide in the cell. Although there are pools of GSH at all times, if they are constantly being used to reduce ROS into water, there could come a point where GSH production is hindered, and in turn, would hinder the activity of GPX. As with SOD, measuring GPX concentration in various sample types could assist in the identification of oxidative stress as it is one of the three major antioxidant molecules responsible for metabolizing ROS. Along with GPX, CAT is also successful in reducing hydrogen peroxide into water and utilizes GSH to do so. Although they both have similar functions, GPX has been found to have a higher affinity for hydrogen peroxide than CAT (Lushchak, 2012). This higher affinity for hydrogen peroxide also supports the idea of utilizing GPX as a marker of oxidative stress.

1.3.3 Glutathione

Glutathione is a vital intracellular and extracellular protective nonenzymatic antioxidant molecule that can solely interact with ROS to produce less lethal products (Zitka et al., 2012) and also act as a ancillary factor to both GPX and CAT. In conjunction with the antioxidant defense system, GSH and GSSG (the oxidized form) are also involved in nutrient metabolism. Noted in figure 1-3, GSH and GSSG work as a coupled reaction. Glutathione is synthesized by γ-glutamylcysteine synthetase and glutathione synthetase utilizing 2 GSSG molecules (Lushchak, 2012; Zitka et al., 2012). Without the presence of GSSG, GSH would not be in the cell. GSH is considered to be the most abundant and ingenious antioxidant found (Espinosa-Diez et al., 2015). Its involvement in other pathways explains the need for GSH to have a constant presence in the cell. Unlike other antioxidants, GSH is solely created in the cytosol (Lushchak, 2012). About 10 to 15% of the total GSH is found in the mitochondria (Lushchak, 2012), thus

the molecules must be able to easily diffuse across the membrane to reach other organelles. Diffusion may be achieved due to the structure of both the reduced and oxidized form of glutathione.

Glutathione, most notably the GSH:GSSG ratio, would be suitable markers of oxidative stress. In a cell at rest, the ratio of GSH:GSSG is 100:1. It has been reported, under times of cellular toxicity, the ratio is likely to be 10:1 or even 1:1. A lower ratio would indicate that the cell is utilizing more GSH than can be produced by glutathione reductase, a secondary enzyme that is necessary for the production of GSH. If a cell maintained a ratio around 100:1, that would indicate that the cell is not under a state of oxidative stress as less GSH is being used.

1.4 Possible feedstuffs to mitigate negative effects of oxidative stress

An abnormality in the antioxidant defense system can increase the susceptibility of pigs to stress, resulting in decreased performance and reduced immune function (Duthie et al. 1989; Lauridsen et al. 1999). Reduced immune function is associated with an increased incidence of disease prevalence in pigs (Pluske et al., 2018). Use of antibiotics against disease has occurred in the industry for decades. However, antibiotic use in food animals has been brought into question by consumers due to concerns of the potential transfer of antibiotic-resistant genes. Worries around the potential development of antibiotic-resistance genes in the presence of antibiotics has led to alternative measures to combat against bacterial pathogen overgrowth. Yeast-based products in swine diets represent a means to combat against pathogen binding in an alternative method compared to antibiotics (Elghandour et al., 2020). Yeast cell components offer support to the immune system of the animal and, in turn, reduce the effect that oxidative stress has on

pig wellbeing. However, there is no knowledge regarding the effectiveness of yeast cell components in reducing ROS production to obtain a homeostatic balance between ROS and antioxidant molecules. Thus, it would be advantageous to seek ingredients that could further prevent the negative effects that arise from oxidative stress, or even potentially inhibit the occurrence of oxidative stress in the animal. Dietary additives that could be utilized against oxidative stress must possess bioactive compounds, particularly antioxidant compounds, that would assist in reducing ROS levels. Phytochemicals are well known products that produce enzymatic antioxidant molecules for the protection of the host. In conjunction, certain vitamins that are already found in the diet also possess antioxidant capabilities. Further investigation of these products is necessary to identify useful feed ingredients that would assist in reducing the state of oxidative stress.

1.4.1 Phytochemicals

The use of plants as medicine has occurred for centuries. Plants utilized were ones that possessed phytochemicals, or bioactive compounds made by the plant, that would assist in eliminating the illness in question. Plants are able to produce a large number of diverse bioactive compounds or phytochemicals (Altemimi et al., 2017), much of which are created in order to maintain the wellbeing of the plant. Many of these compounds protect against the incidence of oxidative stress. Oxidative stress does not only occur within mammalian cells, it is also has been discovered in plants. Xie et al. (2019) elucidated that there are several different stressors that a plant may experience in their lifetime, including times of drought and extreme temperatures. As in animals, stress is a less than desirable state as it can result in retardation of growth for plants and crops. Antioxidant molecules are just some of the phytochemical compounds that are created by

the host as a means to protects themselves. Figure 1-4 illustrates the various phytogenic compounds found in plant species.

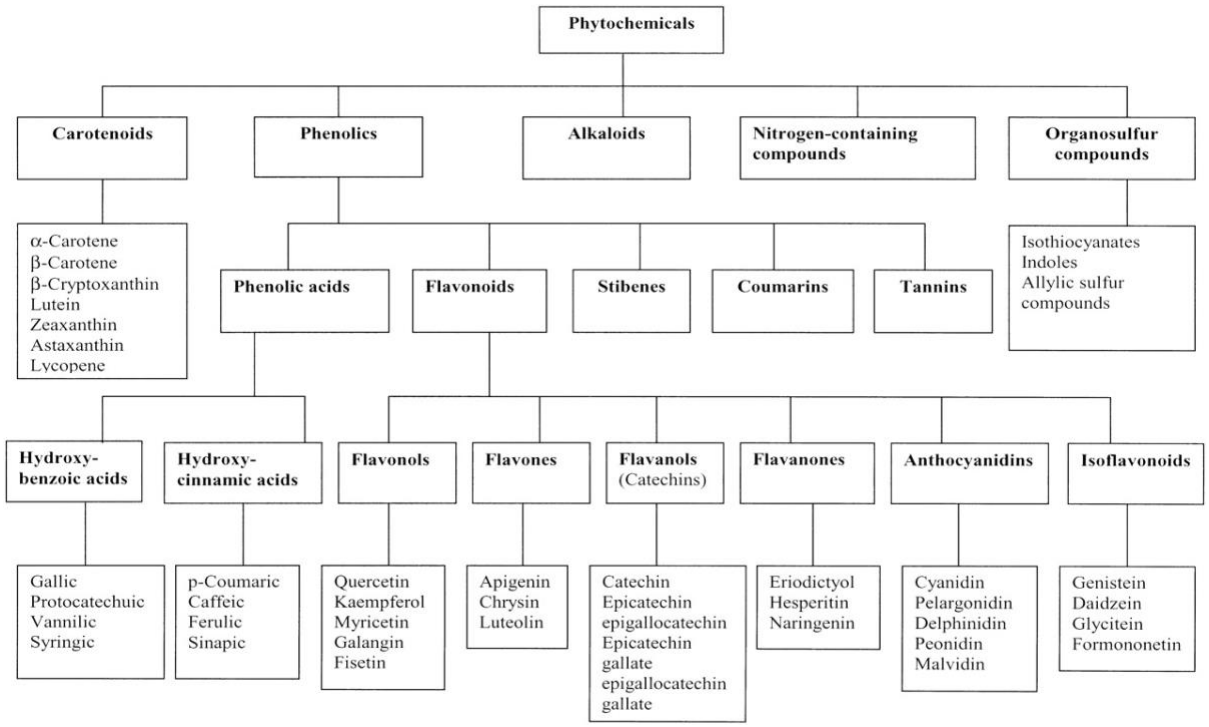

Figure 1.3. Dietary phytochemical classifications [Adapted from Liu (2004)]

Many of these compounds exhibit antioxidant activity, most notably the phenolic class. Phenolic compounds, both natural or synthetic, act as antioxidants or free radical scavengers due to their high reactivity with peroxyl radicals, acting as hydrogen or electron donating agents, and singlet oxygen quenchers and metal chelators (Amorati et al., 2013; Oh et al., 2013).

Physiologically, phytogenic feeds have been reported to decrease ammonia output, oxidative stress and lipid peroxidation in poultry (Windisch et al., 2007) as well as increase overall gut health by supporting symbiotic gut microflora. Phytogenic feed additives (PFA) can also exhibit improvement in performance and health of livestock, due to a reduction in feed intake and improved feed conversion ratio (Settle et al., 2014).

Use of PFA in livestock diets, even in swine, has occurred with most reporting benefits against oxidative stress (Windisch et al., 2008; Bourque et al., 2012; Settle et al., 2014; Tan et al., 2015; Zou et al., 2016; Meng et al., 2018; Lipiński et al., 2019; Reyes-Camacho et al., 2020). Inclusion of phytochemicals in the diet also occurs in the human nutrition industry. Cancer is a notable disease that is characterized by increased ROS production. There is a vast amount of research reporting the effects of phytochemical supplementation in human diets and its ability to decrease cancer risk in patients as illustrated in a review by Rajasekar et al. (2019).

Peppermint is a hybrid species of mint, coming from the breeding of spearmint and Water mint (Riachi and De Maria, 2015). Peppermint exhibits antimicrobial, anti-tumor, and antioxidant activity. Most notably, it is a potent source of antioxidant molecules to the plant and consumer of the plant. Peppermint is mostly composed of terpenes, particularly monoterpenes (Riachi and De Maria, 2015). Bassolé et al. (2010) observed that the most abundant of those monoterpenes, were menthol (39.3%) and menthone (25.2%). The peppermints (Mentha × piperita L.) and two species of spearmints, ‘Scotch’ spearmint (Mentha × gracilis Sole) and ‘Native’ spearmint (Mentha spicata L.) are among the most important crops in essential oil (EO) production worldwide and have been widely used as flavors in food, toothpaste, pharmaceuticals and cosmetics (Bensabah et al., 2013). These products are liquid mixtures of volatile compounds that are commonly collected through steam distillation of aromatic plants (Wu et al., 2019a). Interestingly, oil from peppermint leaves are reported to have a stronger antioxidant activity than the plant itself, due to its scavenging, hydrogen donating, chain breaking, and electron donating capabilities, compared to spearmint and

scotch spearmint (Wu et al., 2019a). Inclusion of oils in the diet may be suitable in alleviating ROS concentration in the cell and potentially illnesses related to oxidative stress or heightened concentrations of ROS. Due to the vast amount of antioxidant phytochemical compounds, peppermint EO was found to be an effective alternative short-term treatment of irritable bowel syndrome in humans, of which the effect is considered to be mediated through its antioxidant and anti-inflammatory activities (Khanna et al., 2014). Supplementation of peppermint and its oil in human and animal diets would be beneficial in providing dietary antioxidants that could assist in hindering oxidative stress and mitigate oxidative damage. However, caution should be taken because results vary from to study to study.

1.4.2 Inactive Yeast Cell

Yeast-based products possess antimicrobial and antibacterial activity due to their structural components. As animals under stress may experience reduced immune response capabilities, the ability to fight off pathogens is impeded. Inclusion of additives that inhibit pathogen binding in the gastrointestinal tract of the animal would assist in managing disease prevalence. *Saccharomyces cerevisiae*, the most widely used yeast strain in the industry, is a common binding additive in swine diets (Elghandour et al., 2020). In conjunction with binding, inclusion of yeast products in swine diets has been noted to improve pig performance as a probiotic or prebiotic (Bass et al., 2019). Several studies have reported beneficial effects of β-glucans, which are a cell wall component of *Saccharomyces cerevisiae* and other yeast, on growth performance and health in pigs (Liu et al., 2017). However, the effectiveness varies from product to product. Many yeast additives in animal production are derived from *Saccharomyces cerevisiae*, however,

there are several other strains that are being introduced. *Pichia guilliermondii* differs in morphology and surface size, but still possesses the ability to bind to pathogenic bacteria. Peisker et al. (2018) observed that *Pichia guilliermondii* inhibited the adherence of pathogenic strains of *Escherichia coli* by 60 to 80% and *Salmonella enterica* by 60-75% in small intestinal epithelium of swine. An ability to bind more than half of the pathogenic strains of bacteria that are found in the intestinal lining is advantageous as it reduces the incidence of sickness in the pig. Similar binding ability of *Pichia guilliermondii* to *Saccharomyces cerevisiae* may suggest that there are other yeast strains that could also be used in the diets of livestock, in particular swine diets.

Although antioxidant molecules are the catalyst in the reduction of ROS concentration, pathogen binding and immunomodulation that occurs with the inclusion of yeast is due to antimicrobial and antibacterial characteristics that the organism possesses. Yeast cells express limited pools of antioxidant molecules which sufficiently protect themselves against ROS (Farrugia and Balzan, 2012). Although yeasts have their own reservoir of antioxidant molecules for protection against oxidative stress, it is unknown if the concentration would be sufficient to provide antioxidant protection to the pig. It is also undetermined if the antioxidant pools would still be present in some yeast-based products after processing.

1.4.3 Vitamin Stereoisomer supplementation

Vitamin E is a subset of the isoprenoid class and its structure comprises a chromanol base with a saturated 16 carbon phytyl chain. A total of 8 stereoisomers are identified for vitamin E which categorizes it as a tocochromanol (Reboul, 2017). Those 8 stereoisomers are α, β, γ, and δ-tocopherol and α, β, γ, and δ-tocotrienols (Figure 1-4).

Saturation of the 16-carbon chain is what determines what is a tocopherol or tocotrienol, with the number and position of a methyl group determining its further classification. Alpha tocopherol (AT) is the most bioactive compound of vitamin E, with the tocotrienols being the least active.

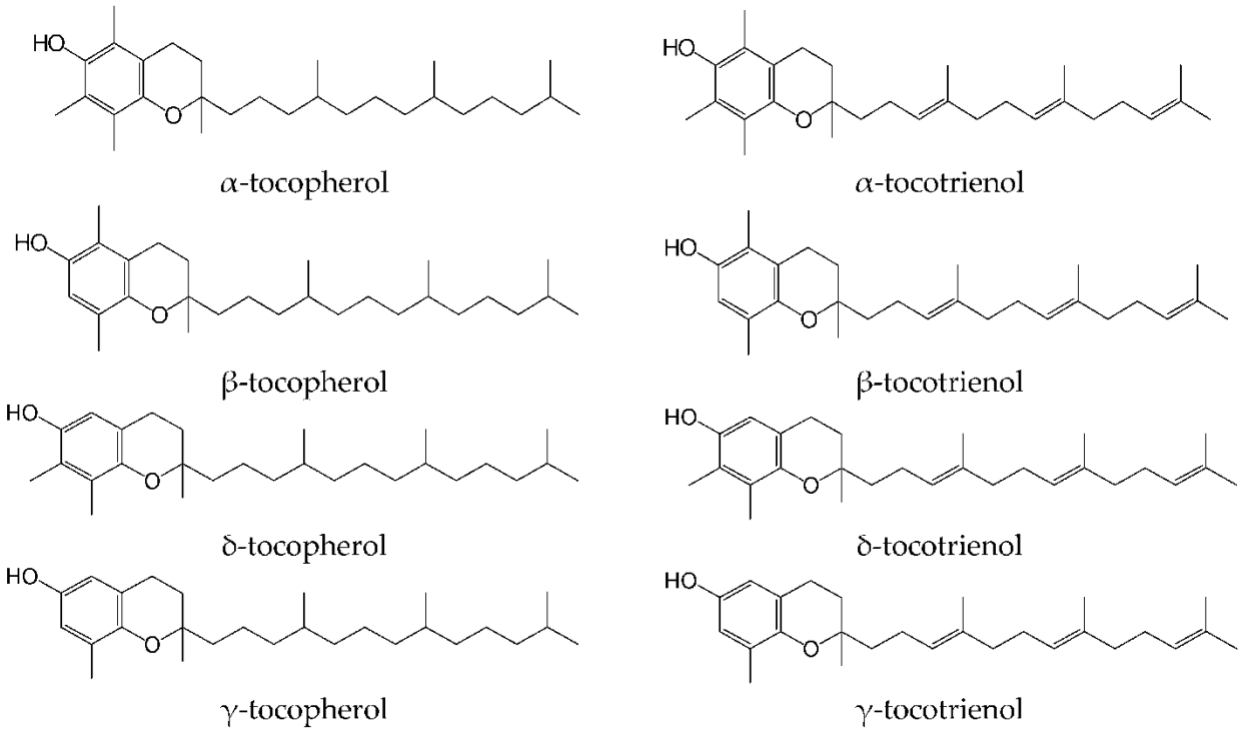

Figure 1.4. Chemical structure of the 8 stereoisomers of Vitamin E [Retrieved from Reboul (2017)].

Vitamin E is an essential nutrient as mammals lack the ability to synthesize this lipophilic antioxidant molecule. Plants or other photosynthetic organisms are the sole organisms that can synthesize vitamin E. Reboul (2017) denotes that vitamin E and its stereoisomers are able to be passively diffused across the basal lateral membrane following emulsification of the lipid phase that houses the vitamin. Interestingly, it has been identified that absorption of α-and γ-tocopherol is mediated by scavenger receptor class B type I (SR-BI; Reboul et al., 2006). This receptor is necessary as it appears that

not all of the stereoisoforms easily diffuse across the membrane. Maternal transfer of vitamin E and its isomers via the placenta is minimal indicating that the acquisition of vitamin E for offspring primarily occurs from milk (Pinelli-Saavedra and Scaife, 2005; Reboul, 2017). As well, if maternal diet is inadequate in vitamin E concentration, then insufficient amounts are absorbed and the erythrocyte is a key target tissue susceptible to oxidative damage, which leads to anemia (Niki and Traber, 2012). This is true for the dam, but also her offspring.

Niki and Traber (2012) note that antioxidant capacity of the different stereoisomers does vary, with alpha being the most effective. There has been extensive research, in both human and animal nutrition, of the effects of α-tocopherol supplementation in the diet (Chawla and Kaur, 2004; McAnulty et al., 2005; Pinelli-Saavedra and Scaife, 2005; Ho et al., 2013). Although most of the composition of Vitamin E is α-tocopherol, there still is a number of tocopherols and tocotrienols that have yet to be researched to determine their benefits in the diet. Gamma-tocopherol (GT) is the second most abundant isomer that makes up vitamin E. Although it possesses a similar structure to the α form, what differs is the number of methyl groups present on the chromane double ring structure. In comparison to the number of studies on supplementation of α-tocopherol, the number of studies looking at solely γ-tocopherol is minimal. Devaraj et al. (2008) found that supplementation of AT, GT, or a combination were effective in reducing the concentration of plasma lipid peroxides, malondialdehyde (MDA), and 4-hydroxynonenal (HNE) in human subjects. This is a very interesting finding as it suggests that γ-tocopherol may be as effective as a dietary antioxidant in fighting oxidative stress within the cell or mammal. The need to determine the

effectiveness of γ-tocopherol alone is necessary as it is the most prominent form found in the diet (Scholl et al., 2006).

1.5 Oxidative stress in the sow

Sows currently used in the swine industry are more hyper prolific due to genetic selection, resulting in increased numbers of piglets born alive per litter. Prolific females experience systemic oxidative stress during late gestation and lactation as a result of increased metabolic burdens placed on the dam (Berchieri-Ronchi et al., 2011;Tan et al.,2015). The exponential growth of fetuses during the second half of gestation results in increased metabolic stress as the dam must supply necessary nutrients for fetal weight gain. An increase in nutrient requirements in late gestation has been reported (Levesque et al., 2011; Samuel et al., 2012; Solà-Oriol and Gasa, 2016; Goncalves et al., 2016; Mallmann et al., 2018, 2019). Conventional feeding program utilized in swine industry may be inadequate in providing sufficient nutrients to the female during late gestation and lactation when the demand is at its highest. Poor nutrient supply will result in a catabolic state in the dam as she provides nutrients to growing offspring. A catabolic state is even more likely in the context of modern sows where the demands to yield sufficient volumes of milk has increased due to increased litter size.

Lactogenesis is the onset of milk secretion and involves changes in mammary epithelium necessary for milk yield (Neville et al., 2001). Divided into two phases, lactogenesis I is thought to occur a few days prior to the onset of birth with the second phase commencing a few days after. The first phase of lactogenesis encompasses varying levels of development in the female, including the differentiation of mammary cells from immature to mature and the production of colostrum components or colostrogenesis

which involves the transfer of IgG from the maternal circulation to mammary secretions prior to the onset of farrowing (Jönsson, 1973; Barrington et al., 2001). Differentiation of mammary glands and colostrum synthesis, in conjunction with the process of parturition, results in amplified stress during this period which could result in oxidative stress. Lactogenesis II encompasses the onset of copious milk secretion (Farmer et al., 2006) and terminates once offspring are weaned, halting underline stimulation. Progression of lactation is met with a period where the sow is unable to consume enough nutrients to match milk yield, leading to a negative energy balance. This negative balance may result in mobilization of body reserves in an effort to meet milk yield demands. As well, the metabolizable energy intake (MEI) from lactation diets throughout lactation is not sufficient to support the energy need of milk production resulting in sow body tissue mobilization (NRC, 2012). Thus, during the lactation period (3–4 weeks), sows can lose up to 25 kg BW, which could represent 10% of their total BW (Cozannet et al., 2018). Within these catabolic conditions, ROS production increases. Stress experienced during these key periods of production is inevitable, however, with the modern high yielding sow, the stress that occurs may be considered to be on a different scale compared to less productive sows as litter size has changed over the decades.

In the livestock industry, oxidative stress has been recognized as one of the major threats to animal welfare, productive performance and the quality of animal products (Fellenberg and Speisky, 2007; Liang et al., 2015; Shurson et al., 2015). For the gestating and lactating dam, diminished performance is less than desirable as it does not solely impact the female. Oxidative stress can also impact the lifetime growth performance of the offspring. Recognizing periods in the sow’s life where oxidative stress is more likely

to occur could assist producers in identifying practices that could help prevent the negative effects of oxidative stress.

1.6 Gestation Oxidative Stress

Increase in metabolic activity during gestation is thought to coincide with an increase in free radical production (Harma et al., 2005), where late gestation has been identified as the point at which the occurrence of oxidative stress is at its highest (Berchieri-Ronchi et al., 2011). The production of ROS is necessary for signaling pathways, however, excess ROS production in the placenta was shown to alter the maternal-fetus exchanges and delay fetal growth in sheep (Mutinati et al., 2013). Saphier et al. (2013) also denotes that oxidative stress takes part in normal pregnancy development, but is highly involved in pregnancy complications. The fine line between sufficient and overproduction of ROS, with the appearance of oxidative stress, during gestation signifies the ever-present concern of stress on dam and fetal development and health. Even more troubling is that oxidative-stress induced damage has been hypothesized to play a role in spontaneous abortions in pregnant humans (Gupta et al., 2007). Occurrence of abortions in food production animals could result in profit losses from those offspring to be lost.

1.6.1 Fetal Development

Porcine fetal growth accelerates during the second half of pregnancy (Knight et al., 1977; Wu et al., 1999; Pond and Mersmann, 2001). Facilitation of fetal weight gain and mammary gland development to allow for colostrum secretion results in an increased nutrient requirement during late gestation. The extent to which nutrient requirements are not being met determines the need of the sow to mobilize body reserves to support fetal

and tissue growth. In conjunction with fetal development, the creation of free radicals is observed to increase throughout the gestational period with peak ROS production occurring around late gestation. In support of gestation, ROS produced act as regulators of embryonic growth and development (Al-Gubory et al., 2010). Concern arises with overproduction of ROS as it is linked to the occurrence of pregnancy complications. Placental mitochondria are responsible for the generation of ATP and ROS, with both products affecting fetal development and metabolism (Holland et al., 2017). If homeostasis between ROS and antioxidant molecules is maintained throughout pregnancy, no issues with fetal development should arise. Increased oxidative damage to the placenta may negatively affect the growth and health of fetuses, as well as postpartum growth of piglets. This is related to the concept of developmental programming, which states that factors associated with early development, especially within the uterine environment, have long-term effects on subsequent health and performance (Barker et al., 1997).

Increased number of piglets born alive per litter increases the within-litter weight variation at birth and results in increased percentage of offspring being light at birth. Increased number of fetuses also are linked to the rise of intra-uterine growth restriction (IUGR) offspring, which results when the number of developing conceptuses exceeds functional uterine capacity (Foxcroft, 2012). However, lightweight piglets do not only arise as a result of uterine capacity being exceeded. Lightweight offspring and IUGR piglets are associated with increased rates of pre-weaning morbidity and mortality, low feed efficiency, poor growth performance, and even reduced carcass and meat quality (Wu et al., 2006; Alvarenga et al., 2013). Interestingly, it was observed that oxidative

damage to the placenta resulted in an increased incidence of IUGR offspring (Scifres and Nelson, 2009). In conjunction with IUGR pigs at birth, oxidative damage to the placenta has been implicated in the occurrence of birth defects. Offspring born with abnormalities that hinder growth and suckling also have a lower success rate of surviving to weaning. Collectively, this evidence indicates that oxidative stress during fetal growth could negatively impact offspring postnatal performance.

1.6.2 Nutrient supply and progression of pregnancy

With rapid fetal growth occurring in the second half of gestation, nutritionally adequate diets are necessary as maternal dietary nutrients are primarily directed toward support of fetal tissue growth (Trottier and Jonston, 2001). Gestation diets within swine industry are typically formulated to meet the nutritional needs of the dam for maintenance of BW, facilitate BW gain and growth of tissues that are related to reproduction. However, as a means to restrict maternal BW gain, pregnant sows are energy restricted (NRC, 2012). Excessive BW gain in gestation results in farrowing complications and can negatively impact lactation performance. Current practices in industry have dietary nutrients provided at a constant level, where the amount of nutrient provided and consumed are the same during the entirety of gestation. This method may be detrimental as overfeeding gestating females can result in weight gain and impact subsequent reproductive performance. However, incidences of insufficient supply of nutrients, particularly protein, arise during late gestation as protein deposition for the fetus increases rapidly as well as mammary gland growth. It was observed that provisions of insufficient lysine levels in gestation and lactation diets resulted in body protein mobilization and fat content to support fetal growth, litter growth, and milk production

(Yang et al., 2009). Catabolism of fat and protein occurring in order to provide sufficient nutrients or energy to the fetuses result increased free radical production. Therefore, a dam under a constant state of catabolism for an extended period of time is subject to oxidative stress. With oxidative stress, comes oxidative damage, which can hinder or damage fetal growth and milk output. As the modern sow is larger in size and leaner compared to previous generations of production sows, it may be necessary to reevaluate common feeding regimes (Yang et al., 2009). This may either require a completely new formulation or the inclusion of additives that could inhibit negative effects of oxidative stress that arise with the need to mobilize body reserves. Diets containing inadequate amounts of essential nutrients, particularly those that supply precursors or act as antioxidant molecules such as soybean meal and vitamin E, may also indirectly result in oxidative stress by impairing cellular defense mechanisms. (Lykkesfeldt and Svendsen, 2007). For example, nutritionally lacking a metal co-factor would result in a lesser concentration of SOD as the antioxidant molecule cannot be active, allowing the concentration of ROS to increase with the defense system having an inability to detoxify and reduce to controllable levels.

1.7 Lactation

In dairy cattle, a spike in ROS production has been reported in the transitional period from late gestation to lactation (Sharma et al., 2011) due to high energy demand and increased oxygen requirement (Gitto et al., 2002). Free radical production begins to diminish to manageable levels throughout lactation to weaning (Berchieri-Ronchi et al., 2011), however, it is unknown whether ROS concentrations may spike during peak lactation. Once milk secretion occurs, the suckling or milking stimulus promotes its

maintenance, defined as galactopoiesis (Farmer et al., 2006). In sows, it is necessary for the production of milk that lactating females consume adequate levels of energy and protein from the diet highlighting the importance behind females achieving full feed intake within five days post-farrow. However, there comes a point where a dam may be unable to increase feed intake at the same rate as milk production (Koketsu etal., 1997; Vadmand et al., 2015), which results in the sow being in a catabolic state to provide the necessary nutrients to produce milk. Catabolism of body protein and fats may potentially result in ROS production. Oxidative stress can occur with prolonged lengths of a catabolic state. Not only is it detrimental to offspring as milk production is negatively affected, but it is costly for producers. In fact, Strathe et al. (2017) determined excessive body tissue mobilization to be expensive as the body reserve lost by the sow must be reestablished during the following gestation, resulting in an increased feed cost to facilitate body weight gain during non-productive days.

It has been reported that maternal antioxidant molecule levels gradually decline during gestation with a significant drop at day 110 of gestation such that the concentration does not normal until the end of lactation (Berchieri et al., 2011). This observation suggests that lactating females have a deficient antioxidant defense system throughout the lactation period and, thus, an increased susceptibility to oxidative stress and oxidative damage. Sies et al. (2005) observed that oxidative damage can impair biological molecules, such as lipids, nucleotides, and proteins in the body. Oxidative damage to DNA, for example, could potentially lead to alteration of metabolic processes, such as lactogenesis II, and could alter milk production and consequently hinder the growth of offspring.

1.7.1 Suckling period

Colostrum and milk, yield and composition would be improved by reducing oxidative stress in the sow, thus improving the health and growth of the offspring (Shen et al., 2015). At birth, piglets are born with scarce levels of body energy stores (Figure 1-5; Theil et al. 2014) as well as being devoid of serum immunoglobins (Le Dividich et al., 2005; Schmitt et al., 2019). Much of the energy found in the newborn animal, mostly composed of glycogen and some fat, is insufficient in meeting the energy required for maintenance and physical activity during the first days of life (Le Dividich et al., 2005). It is vital that piglets consume colostrum within the first few hours of life to obtain sufficient levels of energy to offset the negative energy balance experienced at birth. Furthermore, adequate colostrum intake (~250 g) has been positively linked with a reduction of pre-weaning mortality rate and improved growth performance until weaning (Devillers et al., 2011; Quesnel et al., 2012). In particular, the importance of colostrum consumption is elevated for IUGR and lightweight piglets as they are considered to be poor eaters, have poor acquisition of passive immunity and poor nutritional status (Yuan et al., 2015). Compared to “normal” piglets, IUGR pigs have been reported to have an even lesser amount of available energy at birth at about 19.5 kJ/kg body weight compared to 175 kJ/kg body weight (Mellor and Cockburn, 1986). With reduced attainment of energy, the incidence of poor growth or death increases for smaller piglets during the lactation period. Following the critical first 24-36 hours of life, consumption of sufficient milk is still needed to support growth and development. If piglets are poor eaters, it could lead to lower body weights and may lead to increased mortality rates. As well, it is unknown whether offspring have a well developed antioxidant defense system at birth,

thus the consumption of antioxidant molecules from milk may be needed. In conjunction, the state of oxidative stress that the piglet is under following birth is heightened as they are transitioning from an intrauterine to an extrauterine environment as well as the sudden usage of their lungs (Castillo et al., 2005; Gaál et al., 2006) which bring an onslaught of oxygen molecules into the animal.

Apart from a sufficient supply of energy to the offspring, colostrum and milk contain bioactive compounds that are essential for the wellbeing of the animal, such as immunoglobins, insulin like growth factors (IGF), and hormones. There are a vast number of IGF proteins, however, the two most important are insulin like growth factor 1 (IGF-1) and insulin like growth factor 2 (IGF-2). Along with IGF-2, there is an abundance of receptors for IGF-1 found in the intestinal lining of neonatal animals (Schober et al, 1990). This may suggest that the presence and binding of IGF-1 could impact the function of the intestines of the piglets in the first few days of life in which growth is vital. Much of the literature concerning the transfer of IGFs to offspring via milk consumption is from ruminants, although there are studies that have evaluated the growth hormone in swine, the degree to which the neonate can fully utilized it is unknown. Xu et al. (2002) discovered that IGF-1 in milk is likely to survive the proximal region of the small intestine. This is promising in that the animals will be able to utilize it.

One of the vital components of colostrum is immunoglobins, more specifically immunoglobin G (IgG) and other immune components that can directly or indirectly influence the immune system of the piglet (Le Dividich et al., 2005). Due to the immature immune system of piglets at birth (Le Dividich et al., 2005), intake of immunoglobins is

essential for the offspring to obtain passive immunity from the dam's immune components. Inadequate consumption in the first two days of life would impair this immunity, leaving the piglet more susceptible to illness by pathogens. Intestinal intake of immunoglobins in the immediate period after birth is transient and nonselective in species such as cattle, sheep, goats, swine and others (Hurley and Theil, 2011). Interestingly, much of the immunoglobulin consumed from milk is expected to be partially or completely digested, however, some portion of the immunoglobulin will remain intact or at least partially intact and capable of binding to an antigen (Hurley and Theil, 2011). It is vital that young animals consume sufficient supplies of colostrum in the first 48 hours of life as the concentration of immunoglobins absorbed starts to decrease as the gut "closes up" as the young animal ages (Blum and Baumrucker, 2008). With this closing of the gut, the nonselective uptake that had initially occurred, is now selective with macromolecules no longer being absorbed by diffusion. As well, the concentration of IgG present in mature milk decreases as lactation progresses with an increased presence of immunoglobin A (IgA) insinuating that passive immunity is maintained throughout the lactation period.

Oxidative stress, more so the excessive concentration of ROS in the body, can potentially transfer from mother to newborn via maternal milk (Erdem et al., 2012). Transfer of excessive ROS, combined with an underdeveloped antioxidant defense system, may increase the susceptibility of pigs to oxidative stress. Reyes-Camacho et al. (2020) reported that phytogenic feeds and their phenolic compounds when supplemented to the sow have the ability to be transferred through colostrum and milk. Phenolic

transfer from mom to offspring is advantageous to prevent performance reduction and increase disease incidence during the suckling period.

1.8 Post-wean period

Weaning is the most important period of stress to newly weaned pigs in the swine industry and it deeply affects gut health and the immune system (Liu et al., 2017). Environmental, social, and dietary changes are known contributors to weaning stress. In concert with impacts on gut health and immune system, weaning stress results in reduced pig health, growth, and feed intake (Campbell et al., 2013) that can ultimately lead to the occurrence of oxidative stress. Although the period of weaning stress is short term, its impact can have lasting effects on the performance of animals up to market.

1.8.1 Early Post-Wean

It is common following weaning for feed intake to drop drastically as pigs transition from an all milk-based diet to that of a grain-based one. Although diets are formulated to include milk-based products to entice pigs to eat and are formulated to be nutrient dense, an impact on growth is observed. Campbell et al. (2013) inform that reduced feed intake in the first week post-wean has resulted in MEI to decrease about 60-70% of pre-weaning milk intake and takes approximately 2 week post-wean to achieve full recovery to the pre-weaning MEI level. Inadequate feed intake resulting in poor weight gain can be costly as Kats et al. (1992) report that average daily gain (ADG) in the first week post-wean can impact subsequent growth performance and days to market (ADG of 226.80 g with a days to market of 173 compared to 0 to 149.69 g with a days to market of 179.2).

Passive immunity acquired via milk slowly decreases following weaning and coupled with an immature intestinal barrier function (Lauridsen, 2019) results in an increased disease risk. Epithelial barrier function is defined by the selective prevention of transport of solutes through the paracellular space on the basis of the size of the molecule and the charge it carries (Rao, 2008). Disruption of barrier function can result in a "leaky gut" which allows toxins, antigens, and pathogens, which normally are inhibited, to pass through the intestinal membrane and be absorbed into the blood system. Tight junction proteins, housed at cell-cell contact sites at the apical end of epithelial cells, regulate leakiness by modulating ion selectively and pore size of the epithelium (Van Itallie and Anderson, 2006; Moeser et al., 2017). Activation or stimulation of the immune system as a result of a pathogen invasion can damage tight junctions, ultimately increasing intestinal permeability. Colditz (2002) denotes that immune-system stimulation enhanced the production of ROS and nitrogen-free radicals in the body. Excess production of ROS, most notably hydrogen peroxide, results in disrupted tight junction proteins. Oxidative stress during weaning as a result of elevated ROS production is associated with impaired growth, gastrointestinal disorders, and increased diseases susceptibility (Boudry et al., 2004).

Genetic selection is discovered to result in increased oxidation production in the weaned pig. As noted, genetic selection over the years has yielded larger, leaner, hyper prolific dams. Offspring from these hyper producing sows have also become leaner over the years. Amadori and Zanotti (2016) report that leaner pigs possess abnormally high serum concentrations of reactive oxygen metabolites corresponding to an oxidative stress level under resting conditions similar to that of human beings during intense physical

exercise. Elevated levels of ROS in the serum may increase the likelihood of newly weaned pigs to fall under oxidative stress. As well, production of ROS in the young animal continues to increase as the pig ages (Buchet et al. 2017). It is unknown whether production of these molecules stabilizes once the pig reaches a certain age. Nonetheless, supplementation of antioxidant rich feedstuff that would reduce ROS concentrations or even the inclusion of antimicrobial ingredients to inhibit pathogen binding and reduce immune stimulation would be advantageous.

Research regarding the prevalence of stressors that would lead to the rise of oxidative stress during the finishing period is minimal. Instances where finishing pigs would experience a disruption in the balance between ROS production and antioxidant ability has been noted when under heat stress (Cui et al., 2016). As well, there is little research that has investigate the longevity of a maternal dietary treatment to combat oxidative stress in offspring past the early post-wean period. Some studies have evaluated the impact of a maternal diet on offspring performance from suckling to market (Shen et al., 2017; Bruhn et al., 2020) but none have investigated potential impacts maternal treatment on oxidative stress in progeny long term. Further information to better understand the impacts that oxidative stress and damage in the pre- and post-natal period on performance during the finishing period is necessary.

1.9 Conclusion

Prevalence of oxidative stress during gestation, lactation, and even post-wean has called for intervention methods that would assist in mitigating oxidative damage. Use of various feed ingredients as suppliers of antioxidant molecules, precursors, or immunomodulators in an attempt to reduce the manifestation of oxidative stress in the

dam and her offspring has occurred. However, the effectiveness of each ingredient varies, and few have investigated long-term effects of offspring growth. Thus, a study was conducted to further elucidate means of mitigating the negative implications that arise with oxidative stress. The study objective was to observe the impacts of including three feed additives (whole yeast cell wall component, peppermint oil, and γ-tocopherol) in gestation and lactation diets on sow performance, sow antioxidant capacity, and offspring performance. It was hypothesized that the inclusion of the feed ingredients would result in the reduction of oxidative stress during high points of metabolic activity, such as late gestation and lactation, in turn mitigating negative effects that impact dam performance and ultimately improve offspring performance during the suckling and the post-weaning period.

CHAPTER 2

2.0 Reducing sow oxidative stress during lactation to improve piglet performance.

2.1 Abstract

A study was conducted to assess the supplementation of a yeast cell wall component derived from *Pichia guilliermondii* and phytogenic oil (peppermint oil) in lactation and weaned pig diets on performance and oxidative status of sows and their offspring. A total of 45 sows and gilts (240.7 ± 38.5 kg BW at d110 ± 1 of gestation) were assigned to one of 4 diets: Control (CON), control + yeast cell wall at 0.2% (YC), control + peppermint oil at 10 ppm (MO), and control + yeast cell wall and peppermint oil (YCMO). Diets were provided from d110 of gestation through weaning. Variables measured included: sow body weight (BW), backfat (BF), feed intake (ADFI), litter characteristics at birth, piglet BW, piglet weight distribution at birth and wean, and serum, colostrum, and milk oxidative status. From the 45 females, a total of 481 piglets (6.25 ± 3.35 kg BW at weaning, d 21 ± 4) were weaned and randomly allotted to pens balanced by weight and litter (6-8 pigs/pen) within maternal diet. Pens of pigs received the same dietary treatment as the sow during the suckling phase for 35 d post-wean in a 4-phase feeding regimen (phase 1, d0-6; phase 2, d7-13; phase 3, d14-19; phase 4, d20-35). Variables evaluated: pig BW, ADG, ADFI, G:F, and distribution across weight categories at each phase change. Oxidative status variables and performance data were analyzed as randomized complete block using the Mixed model procedure in SAS v9.4, with sow and pen as experimental unit and Tukey's adjustment for means separation test. Distribution of pig in each weight category and percentage that changed their category was analyzed using the proc Freq method of SAS, within main effects of YC or oil

supplementation. No effect of maternal diet was observed for sow BW (204.52 ± 43.5 kg) at weaning, piglet birth weight (1.44 ± 0.96 kg), litter size (13.09 ± 6 pig/sow) or sow lactation feed intake (6.32 ± 3.05 kg). Piglets from CON females had lesser gain ($P < 0.05$) compared to the other groups during the suckling phase. Superoxide dismutase activity in sow serum (d110 and weaning), colostrum, and milk was not different among diets and was 39, 59, 45, and 10% lower at weaning than d110 in CON, YC, MO, and YCMO sows, respectively. Milk glutathione content tended to be lower ($P < 0.10$) in MO than YCMO sows with CON and YC intermediate. Oil or YC supplementation did not affect the distribution of piglets at birth and weaning or change to wean. Piglets from YC sows were heavier ($P < 0.05$) than CON with MO and YCMO intermediate at weaning and day 6 post-wean with no difference among groups at day 13 (9.1 ± 0.16 kg), 19 (11.3 ± 0.19 kg) and 35 (19.7 ± 0.35 kg). Overall, daily gain was greater ($P < 0.05$) for CON-fed pigs than YCMO pigs. Overall feed intake was greater ($P < 0.05$) for YCMO than MO, but due to lower gain, YCMO-fed pigs had lower ($P < 0.05$) gain:feed (G:F) compared to CON and MO with YC-fed pigs intermediate (0.538 vs 0.617, 0.621, and 0.585, respectively). Inclusion of Citristim or peppermint oil in lactation diets resulted in enhanced suckling piglet growth, which may be related to lower sow antioxidant status. Performance was also improved during the early post-wean period due to postnatal exposure and the supplementation of feed additives in the early post-wean period.

Key Words: Lactation, offspring performance, oxidative stress, weaned pig

2.2 Introduction

Occurrences of stress is not uncommon in an animal's life. Many different factors or situational instances are linked to the manifestation of stress. For mammalian females, lactation is a period of high stress. Mammary glands have a high metabolic rate during lactation (Bauman, 2000) and as a means to prepare for milk production, the glands must undergo changes. Lactogenesis, defined as the onset of milk secretion caused by changes in mammary epithelium (Neville et al., 2001), is the primary cause of elevated metabolic rate. In the swine industry, there has been a push for a more prolific sow in the herd. With increased prolificacy, the number of piglets born per litter has increased. Although larger number born alive is beneficial to increase throughput, there are consequences with this improvement. The main concern is the increased demand on the sow to meet the nutritional needs of her offspring. Lactation diets are formulated to provide enough nutrients for the female to meet her maintenance needs as well as have enough to produce milk for her piglets. However, if the energy provided in the diet is not enough or if the female is not consuming enough feed, the body must undergo tissue mobilization to meet the nutrient shortfall. Body proteins and lipids are mobilized as sources of energy and amino acids that can be used to meet the needs of maintenance and milk production, but when mobilization occurs for an extended period of time, a concern regarding the wellbeing of the female arises. With increased mobilization, it is thought the incidence of metabolic heat stress arises. Although there is little research regarding metabolic heat stress, environmental heat stress is reported to compromise the efficiency of an animal because nutrients are diverted to maintain euthermia as preserving a safe body temperature becomes the highest priority (Baumgard and Rhoads, 2013). It is not unlikely

that the diversion of nutrients to maintain body temperature also occurs with metabolic heat stress. If the occurrence of metabolic heat stress is left untreated, a more detrimental form of stress known as oxidative stress may arise (Kim et al., 2013). Oxidative stress is defined as the imbalance between prooxidant and antioxidants, with the former being produced in vast amounts (Ighodaro and Akinloye, 2018). Oxidative stress is linked to the impairment of DNA, proteins, and even leads to cell death. Damage to DNA in the sow is significantly amplified around d60 of gestation, with elevated damage still maintained throughout the lactational period and still is not completely recovered until the weaning period (Berchieri-Ronchi et al., 2011). Similarly, weaning is a time of stress for young animals and has been linked to the incidence of oxidative stress. If present, performance of the sow and her offspring, pre and post-wean, is assumed to be reduced (Kim et al., 2013; Zheng et al., 2013). Thus, becoming a concern for the producer.

Methods of alleviating oxidative stress and its side effects has been extensively researched in different species, as well as food products. Studies using swine have observe the inclusion of phytogenics in lactation diets as a means to mitigate oxidative stress effects have been evaluated (Lipiński et al., 2019; Reyes-Camacho et al., 2020). Results appeared to be effectiveness in altering the antioxidant status in the dam, however, little followed offspring after weaning to observe its impact on nursery performance, leading to more questions left unanswered. Peppermint oil, which comes from the *Mentha* genus, is known to be rich in antioxidant activity. As oxidative stress in involved in the relationship between the production of antioxidants and free radicals, the presence of a feed additive that could supply exogenous antioxidants to the body may be beneficial in reducing an elevated oxidative status. The inclusion of yeast cell wall

products in swine diets is not a novel concept. However, there is little research evaluating the impact of certain strains of yeast on the reduction of free radicals in the body or even alleviating the negative effects caused by oxidative stress. An inactive yeast cell wall product, derived from *Pichia guilliermondii* (CitriStim, ADM Alliance Nutrition), has been linked with modulating immune functions (Bass et al., 2019). It could be possible that modifying certain functions of the immune system could aid in reducing oxidative stress in the female or her offspring. As the two feed additives have differing chemical makeups, which may allow for their mode of action to differ, that the inclusion of both ingredients may provide an additive effect on the performance and oxidative state of the sow and offspring.

The objective of this study was to evaluate the impact of yeast cell wall component and phytogenic oil supplementation in lactation and nursery diets on sow antioxidant status and offspring performance during the suckling and early post-wean period.

2.3 Materials and Methods

The experimental protocol was approved by the South Dakota State University Animal Care and Use Committee (17-072A) and followed the Guide for the Care and Use of Agricultural Animals in Research and Teaching (Third Ed., 2010). The trial was completed in two blocks, the first ran from February to April, 2018 and the second occurred from March to May, 2018.

2.3.1 Animals and management

The study was conducted in the farrowing and wean to finish barns at the South Dakota State University Swine Education and Research Facility, Brookings, SD. A total

of 45 multiparous and primiparous females (PIC 1050; 240.71 ± 38.51 kg), across two blocks, were used in a 2 x 2 factorial treatment design from d 110 ± 1 of gestation up to weaning (21 ± 2 d of lactation). Sows and gilts were moved into the farrowing room approximately five days prior to parturition and housed in individual farrowing crates (1.83 m x 2.43 m). Feed was dispersed by an electronic feeding system (Gestal 3G; Jyga Technologies, Greeley, KS, USA) allowing daily intake up to 20% above the set curve for ad libitum intake. Feed was provided in 6 meals at 3-hour intervals beginning at 0500h daily. If a female was discovered to be a poor eater or would consume the entire allotment plus the 20% overage, amount dispensed at each feeding was decreased or increased, accordingly. Feed orts were removed every other day, weighed and recorded. Water was provided ad libitum. Sows and gilts were supervised during farrowing by a trained technician and the assigned graduate research assistant 24h/d from birth of the first piglet to the last piglet born in the farrowing group. Sows and piglets were checked twice daily by the research unit manager, assistant manager, and graduate research assistant following the completion of farrowing and up until weaning.

A 1 ml intravulval injection of Dinoprost tromethamine (Lutalyse, Zoetis, Pasippany, NJ) was administered at d 116 of gestation to females that had yet to farrow on their expected date. Piglets were weighed, received a 2 mL intramuscular (i.m.) injection of iron dextran (Uniferon 200, Pharmacosmos, Watchung, NJ), 1 mL oral dosage of Ponazuril (Marquis, Merial, Duluth, Georgia), and, if birth weight was under 1 kg, a 0.25 mL i.m. injection of Excede (Zoetis, Pasippany, NJ) at d1 of lactation. Young animals whose weight ranged from 600 to 800 g were give a 1 mL oral dosage of First Pulse D (Ralco, Marshall, MN) along with Excede, iron dextran and Marquis. Litters

were equalized to 12 to 14 pigs within 48 hours by means of cross fostering or removal. Cross fostering occurred within maternal treatment groups only. Removals included piglets that were deemed to be runts (≤ 600g at birth) and fallbacks that were taken off test and reared on milk replacer (Birthright baby pig milk replacer, Ralco, Marshall, MN) using milk decks (Birthright milk deck, Ralco, Marshall, MN). Fallbacks were defined as piglets who appeared small or thin and had an ADG of ≤ 30 g from birth to time of weighing. Animals whose gain ranged from 35 to 70 g were reweighed within three days to determine if placement into milk deck was needed. At d3 of lactation, animals were processed (tail docking, tattooing, and castration) and administered a 1mL i.m. injection of Circumvent PCV-M G2 (Merck Animal Health, Madison, NJ). Young boars who appeared small or thin were processed at 5 to 6 days of age as a measure to prevent further health decline. Individual animals or whole litters identified with scours after d3 were treated with a 1 mL oral dose of Spectinomycin (Spectogard Scour-chek, Bimeda, Oakbrook Terrace, IL) twice daily for two days. Two weeks following the completion of farrowing, all piglets were orally vaccinated with a 1 mL dose of *Escherichia Coli* vaccine (Entero-vac, ARKO Laboratories, Jewell, IA). At weaning, all animals were administered a 1 mL i.m. injection of enrofloxacin (Baytril 100, Bayer, Shawnee Mission, KS) and Circumvent PCV-M G2 (Merck Animal Health, Madison, NJ).

At d 21 ± 2 post-farrow, all piglets were weaned and transferred to the wean to finish barn. Pigs that were reared on the sow for the duration of lactation were allotted to pens within maternal treatment (6 to 8 pigs/pen; 16-17 pens/maternal treatment; 481 total pigs; 6.25 ± 3.35 kg) and received the same dietary treatment as during the suckling phase. Pens were balanced for weight and litter as much as possible. Piglets reared in

milk decks were weaned to separate pens; post-wean performance was not recorded for this group. Feed and water were offered ad libitum. Sulfadiazine-trimethoprim (Equisul-SDT, Aurora Pharmaceutical, Northfield, MN) was provided in the water at a dose of 900 mL to 5 gallons of water through a 1:128 proportioner and supplied 400 mg/mL of active ingredient for one week; an additional week when looseness or watery stool was still apparent in more than half of the room. Liquid aspirin with caffeine was administered in concert with Sulfadiazine-trimethoprim. Individual veterinary treatment was administered where water medication appeared insufficient; medication type, dose, and reasoning for treatment was recorded. Pigs who were removed from the trial due to poor health, death, or euthanized were recorded with date and weight at removal. All pigs and facilities were checked twice daily by trained research unit manager and assistant manager, and by the assigned graduate research assistant during the course of the study.

2.3.2 Experimental design and dietary treatments

Females were randomly allotted to one of the 4 experimental diets (n=10-12 animals/treatment), balanced by BW, BF, and parity. Dietary treatments were as follows: Control (CON), yeast cell wall (YC), Mint oil (MO), and yeast cell wall + mint oil (YCMO). Control was a standard lactation diet formulated to meet or exceed nutrient requirements for sows in accordance with NRC (2012; Table 2.1). YC was added at 0.2% at the expense of corn to the base lactation diet. MO was added to both combination and mint oil diets as a top dress with a carrier top dress to both control and YC. Both MO (set to ensure 10 ppm of active peppermint oil/d; Table 2.2) and carrier top dress were provided at 50 g/d and placed on top of the 0800h feeding. Based on the observations of Wu et al. (2019), inclusion of MO at 10 ppm was sufficient to increase nematode survival

rate in an induced stress environment. One sow from Control and one from YCMO were removed from test due to prolonged feed refusal and non-responsiveness to veterinary treatments that was suspected to be related to dislike of the treatment ‘taste’. Sow daily feed allocation followed standard SDSU lactation feed curve based on sow parity.

Within a 4-phase nursery pig feeding program, all diets were formulated to meet nutrient requirements of weaned pigs (Table 2.3). Length of each phase was as follows: Phase 1, 6d, Phase 2, 7d, Phase 3, 6d, and Phase 4, 16d. Inclusion level of Citristim in the diet was the same as lactation, with both mint oil and carrier included as a fat blend mix at 0.1% of the diet. Phases 1 and 2 were provided in pellet form with phases 3 and 4 as meal. Water was provided ad libitum.

2.3.3 Data collections, chemical analyses and calculations

Sow BW was recorded at d 110 of gestation, within 24 hours of parturition, and at weaning. Back fat (BF) at the last rib was measured at d 110 of gestation and weaning using an ultrasound (Ibex pro, E.I. Medical Imaging, Loveland, CO). In concert with BF, blood samples were collected via jugular venipuncture into a nonheparinized blood collection tube (BD Vacutainer, Franklin Lakes, NJ) and stored at 5°C to be later analyzed for antioxidant activity. Litter characteristics (total born, born alive, stillborn, mummies, gender distribution) were recorded 24 hours following parturition. Feed orts were weighed and removed every 3 d for determination of sow feed disappearance. Following the completion of the trial, subsequent breeding and farrowing characteristics were evaluated.

Piglets were weighed within 24 hours of farrowing and at weaning. At each weigh period, BW of the population across both blocks was compiled and used to establish three

weight categories: light, average, and heavy. Determination of categories was done with the use of quartiles. Offspring whose weight was at or under 1.1 kg at birth were identified to be associated with an increased risk of preweaning mortality (Feldpausch et al., 2019), thus allowing for the cutoff of the lightweight category to be established. Weight distribution at birth were as follows: low (<1.11 kg), average (1.11 – 1.67 kg), and heavy (> 1.67 kg) weight. At weaning, the weight range for the categories were: low (<5.46 kg), average (5.46 – 6.95 kg), and heavy (>6.95 kg). "Change to wean" was defined as the weight category change of from weaning compared to birth to determine the percentage of animals that maintained, went up or fell back a category.

At each phase change, BW of weaned pigs was recorded. In conjunction with weighing, feed disappearance was documented and ADG, ADFI, and G:F was calculated. Similar to the suckling phase, weights of the two blocks were assigned to one of three weight categories at each weigh period.

Feed samples were collected during lactation and the post-wean period. A pooled sample was collected at each phase for each experimental diet during the post-wean period. Feed samples were ground, placed into whirl-pak bags (Nasco, Fort Atkinson, WI) and shipped for analysis. Diets were analyzed for CP, moisture, ash, ether extract, and crude fiber (Experiment Station Chemical Laboratories, University of Missouri – Columbia).

Following the birth of the first piglet and prior to suckling, colostrum was collected using gentle stripping from all teats for a total volume of 25 ml in sterile conical tubes (Fisher Scientific, Pittsburgh, PA). At d 4 to 5 of lactation, a milk sample was collected; piglets were removed from the sow for one hour followed by a 2 mL i.m.

injection of oxytocic principle (Oxytocin, Aspen Veterinary Resources, Liberty, MO) and gentle stripping of all teats for a total volume of 40 mL in sterile conical tubes (Fisher Scientific, Pittsburgh, PA). Piglets were returned to the dam following collections. Colostrum and milk were stored at -20ºC until further use.

As a means to assess oxidative status in serum and milk samples, three antioxidant markers were selected to characterize each level in the antioxidant defense system. Those markers were: SOD, GSH, and GPx. SOD enzyme concentration was determined by a commercially available kit (Superoxide Dismutase Kit, Cayman Chemical, Ann Arbor, MI), which utilized tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. Within 24 hours of collection, blood samples were centrifuged at 28,000 x g for 20 minutes. Serum was collected and transferred to 1.5 mL microcentrifuge tubes (Thermo Fisher Scientific, Waltham, MA) and stored at -80ºC if not used immediately and between assay runs. Prior to analysis, serum was diluted at 1:10 and colostrum and milk diluted at 1:100 or 1:200. Sera samples were analyzed in duplicates following the manufacturer's instructions. Colostrum and milk sample were analyzed following manufacturer's instructions with the inclusion of a sample blank to correct for noise. Following several attempts to measure GSH in serum samples, it was discovered serum concentrations were below detectable limits thus only GSH in milk is reported. Milk GSH was assessed using a commercially available kit (Glutathione Assay kit, Cayman Chemical, Ann Arbor, MI). As both serum and milk contain large amounts of proteins, there is a possibility that particulates or sulfhydryl groups in protein can interfere with assay results. Thus, samples were deproteinated in accordance with manufacturer's instructions. An equal volume of Meta-Phosphoric Acid

(# 239275, Sigma-Aldrich, St. Louis, MO) reagent and sample was combined and vortexed to mix. Following an incubation time of 5 minutes at room temperature, tubes were centrifuged at 4,000 x g for 4 minutes. The supernatant was divided equally between two or three microcentrifuge tubes; samples were stored at -80ºC until analyzed. This method of deproteination resulted in only total GSH content to be measured. Prior to analysis, TEAM reagent (50 µl 4 M triethanolamine per 1 mL supernatant, # T58300, Sigma-Aldrich, St. Louis, MO) was added to one of the subsample tubes and tubes vortexed.

In conjunction with antioxidant status, colostrum and milk samples were analyzed for protein, lactose, total solids, and fat (Division of Regulatory Services, University of Kentucky, Lexington, KY). Milk composition was measured in an attempt to determine if supplementation of feed additives would alter the chemical makeup of milk.

2.3.4 Statistical analysis

Data was analyzed using the mixed model procedure of SAS (Version 9.4, SAS Inst. Inc., Cary, NC) considering the effect of dietary supplementation where the sow was the experimental unit and sow (block) as the random effect during the farrowing and suckling period. In the post-wean period, pen was the experimental unit with pen (block) as the random effect. Variables of particular interest included sow reproductive performance (i.e. litter size, lactation feed intake), sow antioxidant status and piglet performance (i.e. nursery feed intake, growth rate during lactation and in the nursery). Significant differences were reported at $P < 0.05$ and tendencies for significance were reported when $0.05 \leq P \leq 0.10$.

Weight categories were analyzed using the Freq procedure in SAS (Version 9.4, SAS Inst. Inc., Cary, NC). An interaction between dietary treatments or weight category was not detected thus data was analyzed as main effects of base (Control and Citristim) and oil (carrier and mint oil) separately using the CATMOD procedure in SAS (Version 9.4, SAS Inst. Inc., Cary, NC).

2.4 Results

2.4.1 Diet Analysis

Table 2.4 and 2.5 report AA and proximate analysis of lactation and nursery diets (Phase 1, 2, 3, and 4), respectively. Amino acid and CP content of YC lactation diet appeared to be 8–11% below CON, with both within the expected analytical ranges of the formulated values.

Farrowing and Suckling Growth Performance

Sow BW in gestation, lactation, and weaning were not affected by maternal dietary treatment (Table 2.6). Backfat at trial start was lower ($P < 0.05$) in females assigned to YC compared to females assigned to MO and YCMO. A similar pattern was observed at weaning where backfat of YC females tended to be lower ($P < 0.10$) than YCMO females and CON and MO females intermediate. Sow lactation feed intake was not affected by dietary treatment. Litter born alive, stillborn, mummies and piglets weaned was not affected by treatment. It is interesting to note that 13.5% of pigs were removed from the MO group and 15 – 18% were removed from the other groups (Table 2.6). No effect of maternal dietary treatment was observed for birth weight. Piglets from CON females were lightest ($P < 0.05$) at weaning due to lesser daily gain ($P < 0.05$) compared to piglets from all other groups. However, there was no difference between

YC, MO, and YCMO piglets. While growth of artificially reared piglets were not followed past weaning, inclusion of these pigs in the sucking performance data, difference by maternal dietary treatment remained as described above. Total kg of pig in each litter based on pigs suckling was calculated. Even though MO offspring had a similar birth weight as YC, MO dams appeared to wean more kg of pigs.

Offspring weight distribution at birth or weaning was not different between groups (Table 2.7). No difference in change to wean was detected although 27-31% of offspring fell back a category and 12-18% went up with either base or oil top dress. Interestingly, offspring from sows provided the MO top dress had about 4% less fall back and with 4% more move up one category compared to those given the carrier top dress. Piglets that were removed included those that were runts at birth or those that became fallbacks during the duration of the trial, as well as those that died. There was no difference in the percentage of piglets removed, although there did appear to be slightly more piglets removed from sows fed YC which may have influenced change to wean.

2.4.2 Post-wean Growth Performance

Pigs fed YC were heaviest ($P < 0.05$) and pigs fed YCMO were lightest ($P < 0.05$) at d6 post-weaning with CON and MO intermediate (Table 2.8). At d13 post-weaning, pigs fed YCMO tended to be heavier ($P < 0.10$) than CON pigs. There was no difference in pig BW at d19 and 35. Daily gain was greater ($P < 0.05$) in pigs fed CON than YCMO in the first 6d post-wean. Although an effect of treatment on daily gain from d7 to 13 was reported, there was no between treatment groups difference based on Tukey's adjusted means test. Gain in the last phase (d20-35) was lower ($P < 0.05$) in pigs fed YCMO compared to pigs fed CON. Average daily feed intake was greatest ($P < 0.05$)

for CON and MO-fed pigs in Phase 1, greatest ($P < 0.05$) for CON followed by MO-fed pigs in Phase 2, greatest ($P < 0.05$) for both groups fed MO in Phase 3, and greatest ($P < 0.05$) for CON-fed pigs in Phase 4 with no difference between pigs fed YC or MO. G:F was lowest ($P < 0.05$) in YCMO and highest ($P < 0.05$) in MO-fed pigs in Phase 2, lowest ($P < 0.05$) in YCMO-fed pigs in Phase 3, and not different between treatments in Phase 4. For the entire period, pigs fed the YCMO diet had the lowest G:F ratio ($P < 0.05$) compared to CON and MO pigs.

Given the response in the suckling period, the distribution of light and heavy weight weaned pigs was the emphasis in the post-weaning period (Table 2.9). Up to d19 post-weaning, majority of the lightweight pigs remained within the lightweight category for all treatment groups. There was a tendency ($P < 0.10$) for a greater proportion of light-weight pigs in the MO group at d6 post-weaning. By the end of the trial period 27% of the lightweight MO-fed pigs had moved to the average or heavy category compared to 23% of carrier-fed pigs. In pigs deemed heavy at weaning, a greater ($P < 0.05$) proportion receiving YC diets were assigned to the heavy category at d6 post-weaning and likely reflects the heavier wean weight in this group. A proportion of heavyweight pigs at weaning were observed to fall back and become average or lightweight in the post-weaning period. However, at d35, 53% of YC-fed pigs deemed heavy at weaning were still heavy compared to 33% of CON-fed pigs.

2.4.3 Antioxidant Activity and Composition

Percent fat, protein, lactose, total solids and solids not fat in colostrum was not affected by dietary treatments; similarly, for milk protein, lactose, total solids, and solids

not fat. Percent fat tended ($P < 0.10$) to be lower in YCMO females compared to all other groups (Table 2.6).

No effect of dietary treatments was noted for serum concentration of SOD at d 110 of gestation or at weaning (Table 2.10). There was no effect of treatment on SOD content in colostrum or milk but a reduction in SOD as lactation progressed (i.e. colostrum vs milk) was observed. Similarly, there was no impact of treatment on GSH content in colostrum but a tendency for greater ($P < 0.10$) GSH content in milk from YCMO sows.

2.4.4 Subsequent Performance

In the subsequent parity, sows fed diets containing YC, with or without MO, had an average of 14.5 piglets born alive while sows fed CON with or without the MO top dress averaging 13. Within each of the CON and MO groups there was 1 sow with a subsequent litter size of <8 and 1 with a litter <10 piglets. Because the data is made up of only 7 – 8 sows/group these relatively small litters have a considerable impact on the overall average and hence, subsequent performance may be as likely due to random chance as an effect of supplementation. One group of sows from this study were used in another trial in their subsequent parity, where an additive was included in both gestation and lactation diets. Females were evenly distributed among the subsequent treatments thus any differences in subsequent litter born alive for this group are unlikely related to dietary treatments from this trial.

2.5 Discussion

The purpose of the study was to assess the effect of supplementing Citristim, peppermint oil, or a combination of the two on oxidative status of the sow during lactation and offspring growth from birth to the early post-wean period. Although no

difference was detected for antioxidant status in the dam, an improvement in offspring performance was observed implying that slight modification to dam antioxidant activity still provided benefit to progeny. Based on analytical kits used, a higher SOD value represents greater need of the SOD enzyme for dismutation of superoxide radical and greater free radical insinuating a higher state of stress. Conversely, a lower GSH value insinuates greater oxidative state. SOD activity at d110 of gestation varied numerically, with sows assigned to the YC and MO groups possessing higher levels indicating that the females were at a greater level of oxidative stress. At weaning, the concentration of SOD for all treatment groups were similar in value. This similarity may provide evidence of a greater reduction in oxidative stress in the YC and MO sows and may, in part, explain differences in offspring growth. With respect to maternal diets, the 2 to 4-fold reduction in serum SOD at weaning in MO group may suggest that the incidence of oxidative stress diminishes as the lactation period progresses which seems to be somewhat supported by lower GSH in d4 milk.

Late gestation appears to be a period of greater oxidative stress than lactation, as demonstrated by differences in d110 serum and colostrum vs d4 milk and wean serum, respectively. Although some level of elevated stress could be detected at trial start, this may well be a result of randomization to treatment rather than dietary treatment itself. Wisdom et al. (1991) illustrates that pregnancy is a period of constant oxidative stress for the dam. This period of increased oxidative stress may be a result of reproductive stress. Reproductive stress is defined as the non-specific response of the body to reproductive activities including the estrous cycle, pregnancy, parturition and lactation (Wen et al., 2019). Increased fetal growth in the second half of pregnancy with mammary

development may be contributors to this reproductive stress. Lactation is a period of elevated stress in lactating females and it is theorized that at peak lactation the level of stress is at its highest. During peak lactation, the nutrient demands to meet both maintenance and milk production is at its maximum. However, when energy intake is insufficient to support the maintenance energy requirement and milk production, then both maternal body lipid and protein are mobilized and used as sources of energy (NRC, 2012). A constant state of mobilization could be linked with an increased incidence of oxidative stress. It is estimated that sows would be back on feed around d3 or 4 of lactation following farrowing. With increased intake, the energy demands for both maintenance and milk production are believed to be met. Thus, the need to mobilize body reserves are lessened, reducing the incidence of oxidative stress or production of free radicals in the dam. Milk samples collected at d4 of lactation observed that the concentration of SOD decreased and GSH increased compared to the concentration in colostrum. This change of concentration throughout the lactation period could confirm that there is a lower rate of body reserve mobilization occurring. If samples were collected around the time of peak milk production, it could be theorized that the concentration of both antioxidant markers would be similar to that of colostrum.

Inclusion of both test ingredients did not offer an additive effect on the performance of lactating sows or offspring during the suckling and early post-wean period. CitriStim, which is derived from *Pichia guilliermondii,* possesses antimicrobial activity allowing for the binding of pathogens in the small intestine and has been reported to modulate immunomodulatory effects in broilers (Shanmugasundaram and Selvaraj, 2012). Peppermint oil, or *Mentha piperita*, is a part of the *Mentha* family which is known

to possess antioxidant activity. Some report that this species of *Mentha* can induce apoptotic cell death in *S. cerevisiae* (Ferreira et al., 2014), however, as *S. cerevisiae* and *P. guilliermondii* have different morphology it is unsure if the same would be true with *P. guilliermondii*. If true, then peppermint oil could have the ability to cause apoptotic cell death in *P. guilliermondii* thus eliminating antimicrobial benefits that the yeast strain would have offered. With no additional support provided, performance would be the same to females supplemented peppermint oil only. This seems likely as some of the piglet performance data in the suckling period for both the peppermint oil and combination diet were close in value.

Bass et al. (2019) noted that the inclusion of Citristim in gestation and lactation diets did not improve piglet birth and wean weight. Results from this current study also did not detect a difference in birth weight between YC and control-fed sows. While a difference was not expected for birth weight given the diets were implemented a few days prior to farrowing, wean weight and suckling period gain of pigs reared on YC sows were heavier than control. However, smaller number of pigs suckling, and hence weaned, from YC-fed sows may also have contributed to the greater growth in this group. In a study that evaluated the effects of oregano oil on sow oxidative stress status and piglet performance, it was reported that the inclusion of the test ingredient in gestation and lactation diets did not result in a difference in colostrum and milk composition (Tan et al., 2015). This is in agreement with the current study where colostrum composition was not different. The mechanism for difference in fat content in YCMO-fed sows is unclear.

Dietary treatments in the post-wean period resulted in pigs from the YCMO diet to gain less in the entire trial period as well have a lowest F:G ratio compared to CON

and MO. Interestingly, pigs supplemented MO had a G:F ratio comparable to CON pigs. Weaning is known to be a time of high stress for young animals due to the sudden dietary, environmental, and social changes. With newly weaned animals, the occurrence of oxidative stress has been noted to result in reduced growth performance, increased disease incidence and even death (Zheng et al., 2013). These outcomes are related to the denaturing of tight junction proteins lining the small intestine as a result of the over production of free radicals. With reduced barrier function of the intestinal epithelium as a result of disrupted tight junction proteins, the potential for undigested feedstuff and toxins in the intestinal lumen to pass the basal lateral membrane increases. Inclusion of peppermint oil would assist reducing ROS concentration in lipid membranes potentially reducing gut permeability. Coupled with the inclusion of Citristim, which has antimicrobial activity and could bind to pathogens found in the intestinal lumen, the idea that the inclusion of either one or even the combination of the two would aid in the prevention of poor growth in the early post-wean period. However, as observed in the lactation period, combination of the additives diet did not result in a better performance compared to pigs that were given the feed additives separately.

Selection for a more prolific sow has resulted in an increased number born alive per litter (Quiniou et al., 2002) resulting in increased within-litter variation at birth with the proportion of lightweight pigs increasing. It has been reported that heavier birth weight piglets consume about 30% more milk than their lighter littermates (Pluske and Williams, 1996). Interestingly, the inclusion of Citristim provided a means for heavyweight animals at birth and weaning to maintain their heavyweight status. Concern regarding performance during the early post-wean period should not only revolve around

lightweight pigs as results from this study suggest there is an opportunity for heavy pigs to lose their heavy-weight status. Inclusion of Citristim in the diet after weaning, combined with exposure during the suckling period, appears to provide support to heavy pigs in maintaining their heavy weight status. The mechanism for this benefit is unclear. Alternatively, inclusion of peppermint oil offered assistance to lightweight animals during the suckling period. This benefit was also evident during the post-wean period in that the percentage of animals that were deemed light was lesser for those from MO sows. Concern regarding the performance of small pigs in the post-wean period relate to increased labor and feed costs. An increase in feed and management costs was found for lighter pigs, due to the longer time and increased feed required for them to reach the required minimum commercial slaughter weight (Yuan et al., 2015). Variation at weaning and up to slaughter can negatively impact the monetary gain for producers as lightweight animals need more days to market than their heavier counterpart, which results in increased feed cost. This study suggests that ‘opportunity’ pigs, those that are light or borderline average weight, could potentially gain weight to become average and in turn reduce days to market.

2.6 Conclusion

Although the mechanism by which piglet growth is enhanced is unclear, the apparent reduced antioxidant state in lactating females as a result of Citristim or peppermint oil inclusion may be part of the answer. Further, inclusion of Citristim and peppermint oil in post-weaning diets provided benefit to both light and heavy weight animals based on improvement or maintenance of weight during the suckling period. This

suggests that the inclusion of both additives in sow and pig diets could aid producers in reducing costs, labor, and time needed to support fallback or light weight animals.

Table 2.1 Ingredient composition and nutrient content of lactation diet[1].

Ingredients %	**Control**
Corn	66.27
Soybean meal, 46.5%	29.85
Monocalcium phosphate	1.76
Limestone	1.22
Salt	0.5
Sow Vitamin premix	0.05
Trace Mineral premix	0.15
Toxin Binder	0.2
Calculated nutrient content, %	
Dry matter	87.61
Protein	18.58
Crude fat	2.51
ME, kcal/lb	1487.0
Calcium	0.92
Phosphorus	0.70
Phos avail-swine	0.45
Lysine	1.06
Methionine	0.31
TSAA	0.63
Threonine	0.71
Tryptophan	0.24

[1]Citristim was added at 0.2% at the expense of corn

Table 2.2 Ingredient composition and nutrient content of carrier and mint oil top dress.

Ingredients, %	**Carrier**	**Peppermint Oil**
Distillers Dried Grain	98.64	98.64
Peppermint Oil Fat Blend	0	1.36
C:18 Fat Base	1.36	
Calculated nutrient content, %		
Dry Matter	89.32	89.32
Protein	10.68	10.68
Crude fat	8.68	8.68
ME, kcal/lb	1569.0	1568.0

Table 2.3 Ingredient composition and nutrient content of the Control weaned pig diet[1].

Ingredients, %	**Phase 1**	**Phase 2**	**Phase 3**	**Phase 4**
Corn	30.15	35.72	54.20	69.96
Whey	30.00	25.00	10.00	-
SBM	17.57	19.45	27.71	30.35
HP 300	8.00	7.00	-	-
FM	8.00	7.00	3.00	-
Soy oil	4.30	3.70	2.00	-
Monocal	0.44	0.50	1.10	1.41
Zinc oxide	0.42	0.42	0.28	-
Calcium carbonate	0.25	0.32	0.85	1.02
Lysine, HCl	0.25	0.26	0.32	0.40
Methionine	0.20	0.20	0.15	0.15
Threonine	0.11	0.11	0.12	0.15
Tryptophan	0.03	0.03	0.03	-
Vitamin premix	0.05	0.05	0.05	0.05
Mineral premix	0.15	0.15	0.15	0.15
Selenium	0.05	0.05	-	-
Salt	0.03	0.03	0.03	0.35
C18:fat base	0.01	0.01	0.01	0.01
Calculated nutrient content, %				
DM	91.14	90.58	88.78	87.62
CP	23.85	23.34	20.30	19.35
Lysine, total	1.77	1.71	1.46	1.38

[1]In each phase, Citristim was added at 0.2% at the expense of corn to create the Citristim diet, mint oil was added at 0.01% to the Control and Citristim diet to create Control+mint and Citristim+mint diets. The C:18 fat base was included in the Control and Citristim diets at 0.01% to balance fat inclusion across all treatments.

Table 2.4 Amino acid and proximate analysis of the lactation experimental diets.

Items, %	**Citristim**	**Control**
Proximate analysis		
CP	18.66	21.44
Moisture	13.27	13.43
Crude Fat	1.39	1.44
Crude Fiber	1.88	2.22
Ash	5.21	5..02
Amino acid analysis		
Tau	0.18	0.17
Hyp	0.03	0.03
Asp	1.94	2.18
Thr	0.71	0.79
Ser	0.80	0.89
Glu	3.44	3.83
Pro	1.18	1.28
Gly	0.78	0.87
Ala	0.93	1.01
Cys	0.31	0.34
Val	0.90	1.00
Met	0.26	0.28
Ile	0.83	0.93
Leu	1.62	1.76
Tyr	0.57	0.62
Phe	0.95	1.06
Hyl	0.03	0.03
Orn	0.01	0.02
Lys	1.10	1.24
His	0.50	0.55
Arg	1.22	1.35
Trp	0.20	0.25

Table 2.5 Amino acid and proximate analysis of the experimental nursery diets for phases 1, 2, 3, and 4.

	Phase One				**Phase Two**				**Phase Three**				**Phase Four**			
Items, %	CON	YC	MO	YCMO	CON	YC	MO	YCMO	CON	YC	MO	YCMO	CON	YC	MO	YCMO
Proximate analysis																
CP	24.33	23.97	24.89	24.47	23.64	23.76	23.75	23.88	21.32	18.76	18.65	21.03	18.87	20.24	18.99	18.99
Moisture	8.20	7.34	7.77	6.82	7.78	7.55	7.52	7.40	11.23	11.02	11.23	11.19	13.55	14.02	13.69	13.75
Crude Fat	6.43	7.09	6.49	6.22	5.63	5.65	5.65	5.81	3.13	3.31	3.25	3.44	1.23	1.04	1.24	1.69
Crude Fiber	1.70	1.74	1.73	1.61	1.67	1.66	1.54	1.56	1.74	1.64	1.68	2.09	2.16	2.19	2.15	2.30
Ash	7.35	7.44	7.37	7.21	7.22	7.15	6.92	6.88	6.20	5.85	5.44	5.88	4.81	4.79	5.08	4.78
Amino Acid analysis																
Tau	0.15	0.20	0.19	0.20	0.18	0.18	0.18	0.18	0.16	0.16	0.17	0.16	0.13	0.13	0.13	0.13
Hyp	0.19	0.19	0.14	0.19	0.17	0.12	0.15	0.15	0.07	0.04	0.08	0.08	0.02	0.02	0.03	0.03
Asp	2.34	2.46	2.45	2.45	2.34	2.34	2.35	2.33	2.19	2.00	2.02	2.02	1.85	1.90	1.86	1.73
Thr	1.06	1.07	1.12	1.09	1.05	1.07	1.07	1.06	0.98	0.91	0.87	0.87	0.83	0.88	0.89	0.81
Ser	0.91	0.95	0.93	0.94	0.92	0.93	0.93	0.94	0.89	0.80	0.85	0.79	0.84	0.87	0.87	0.77
Glu	3.84	3.98	4.01	3.97	3.87	3.83	3.84	3.79	3.73	3.46	3.45	3.43	3.32	3.38	3.33	3.14
Pro	1.17	1.44	1.40	1.40	1.36	1.31	1.35	1.35	1.25	1.15	1.17	1.17	1.11	1.13	1.10	1.07
Gly	1.11	1.15	1.14	1.16	1.06	1.07	1.07	1.08	0.90	0.84	0.84	0.85	0.78	0.75	0.75	0.75
Ala	1.19	1.22	1.23	1.24	1.17	1.16	1.17	1.17	1.05	0.99	0.99	0.98	0.94	0.93	0.91	0.90
Cys	0.35	0.38	0.38	0.36	0.36	0.36	0.37	0.36	0.35	0.34	0.34	0.32	0.30	0.31	0.29	0.28
Val	1.19	1.22	1.23	1.22	1.16	1.16	1.15	1.15	1.03	0.96	0.95	0.98	0.90	0.90	0.88	0.88
Met	0.57	0.61	0.62	0.61	0.60	0.59	0.59	0.57	0.47	0.52	0.45	0.44	0.42	0.40	0.42	0.38
Ile	1.08	1.12	1.13	1.12	1.07	1.07	1.07	1.06	0.95	0.90	0.87	0.90	0.83	0.83	0.81	0.79
Leu	1.87	1.93	1.94	1.93	1.87	1.86	1.86	1.86	1.79	1.70	1.67	1.66	1.64	1.65	1.60	1.56

Tyr	0.73	0.76	0.77	0.76	0.72	0.73	0.75	0.72	0.70	0.65	0.66	0.64	0.65	0.66	0.66	0.62
Phe	1.03	1.07	1.08	1.07	1.04	1.03	1.03	1.02	1.01	0.95	0.93	0.93	0.93	0.94	0.91	0.88
Hyl	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.02	0.00	0.01	0.02	0.02	0.02	0.02
Orn	0.04	0.05	0.04	0.04	0.04	0.03	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.01	0.01	0.01
Lys	1.69	1.70	1.74	1.72	1.74	1.65	1.67	1.64	1.57	1.39	1.43	1.52	1.42	1.44	1.50	1.45
His	0.62	0.65	0.64	0.65	0.62	0.62	0.63	0.62	0.59	0.50	0.55	0.55	0.49	0.49	0.49	0.47
Arg	1.35	1.43	1.42	1.43	1.36	1.37	1.39	1.35	1.33	1.21	1.23	1.20	1.19	1.21	1.20	1.12
Trp	0.31	0.32	0.33	0.33	0.32	0.36	0.33	0.34	0.27	0.26	0.27	0.26	0.23	0.23	0.23	0.24

Table 2.6 Performance of sows fed lactation diets with or without Citristim and with or without a mint oil top dress.

Items	Control	Citristim	Control Mint oil+	Citristim Mint oil+	SEM	P-value
No. Sow	12[2]	11	11	11[1]		
Parity, n						
gilt	4	4	4	4		
1	0	1	0	0		
2	7	5	6	6		
3	1	1	1	1		
Sow BW, kg						
d 110	242.5	239.1	244.6	246.1	6.3	0.834
Farrowing[3]	225.8	222.5	219.6	229.0	7.4	0.779
Weaning	226.4	213.9	207.6	215.5	12.8	0.673
Sow back fat, mm						
Entry	12.9[x,y]	12.4[y]	15.3[x,y]	15.5[x]	1.0	0.031
Wean	11.1[x,y]	10.4[y]	12.2[x,y]	13.6[x]	0.91	0.085
Change	-1.8	-1.6	-2.9	-1.3	0.73	0.294
Feed Intake, kg/d	6.41	6.59	6.68	6.64	0.42	0.952
Colostrum Composition, %						
Fat	4.53	3.88	4.07	3.92	0.42	0.468
Protein	17.52	16.11	17.09	15.98	0.96	0.570
Lactose	2.85	2.47	2.44	2.67	0.37	0.817
Total Solids	29.23	26.9	28.14	26.8	0.82	0.101
Solids not Fat	22.64	21.03	21.96	21.07	0.68	0.243
Milk Composition, %						
Fat	7.75[x]	7.15[x]	7.23[x]	6.01[y]	0.56	0.084
Protein	5.15	4.8	4.91	5.07	0.13	0.218
Lactose	5.34	5.33	5.46	5.5	0.08	0.337
Total Solids	19.3	18.3	19.7	17.6	0.64	0.156

Solids not Fat	10.8	10.5	10.7	10.8	0.14	0.247
Litter, average						
Born Alive	14.08	13.82	14.82	13.82	0.87	0.831
Stillborn	0.92	0.72	0.91	1.27	0.33	0.706
Mummies	1.1	0.97	1.32	0.93	0.34	0.784
Weaned, all	12.76	12.59	14.02	13.00	0.80	0.526
Weaned, suckled	10.91	10.02	11.48	10.03	0.93	0.325
Piglets, number						
Born alive	169	152	163	152		
Stillborn	11	8	10	14		
Mummies.	10	7	11	7		
Weaned, all	153	139	154	143		
Died	16	13	9	9		
Birth deck	16	18	18	28		
% removed[4]	18.9	20.4	16.6	24.3		
Piglet BW, kg						
Birth	1.46	1.44	1.39	1.39	0.05	0.729
Wean, all	6.16^{b}	6.65^{a}	6.40^{ab}	6.17^{b}	0.15	0.006
Wean, suckling	6.45^{b}	6.93^{a}	6.71^{ab}	6.56^{ab}	0.16	0.013
Kgs weaned/sow, suckling	67.7	67.7	75.3	64.2	4.3	0.288
ADG, kg/d, all	0.22^{y}	0.23^{x}	0.22^{x,y}	0.22^{x,y}	0.005	0.096
ADG, kg/d, suckling	0.23^{b}	0.24^{a}	0.23^{ab}	0.24^{ab}	0.006	0.041

[1]One sow was removed from the trial due to feed consumption issues in lactation and refusal to nurse piglets. Litter data is included in birth deck count and 'all' performance.

[2]One sow and litter (n=11 piglets) were weaned 4d early due to feed refusal. Two piglets were weaned to the birth deck, the remaining 9 were given milk replacer and creep pellets in the crate and transferred to the nursery along with the other weaned pigs.

[3]Females were weighed within 24 hours of parturition

[4]Includes pigs that died and fall backs/starveouts moved to birth decks.

Table 2.7 Distribution (%) of suckling piglets in weight categories[1] and removed (birth deck, died).

	Control	Citristim	Chisq	Carrier	Mint oil	Chisq
Birth weight range, kg						
0.65 to 1.1	9.1	8.3	0.860	9.3	8.2	0.821
1.11 to1.67	66.7	65.7		65.0	67.4	
1.68 to 2.46	24.2	26.0		25.7	24.4	
Wean weight range, kg						
3.4 to 5.45	28.4	24.8	0.338	27.9	25.6	0.204
5.46 to 6.95	48.8	47.1		50.2	45.7	
6.96 to 10.1	22.8	28.1		21.9	28.7	
Change to wean[2]						
-2	2.8	2.1	0.831	2.2	2.7	0.350
-1	30.9	26.9		30.9	27.1	
0	50.9	54.9		54.2	51.2	
1	15	15.7		12.3	18.6	
2	0.4	0.4		0.4	0.4	
Piglets removed[3]						
Birth deck/dead	14.1	18.2	0.159	16.2	16.0	0.949
Sow	85.9	81.8		83.8	84.0	

[1]Weight categories based on mean ± 1 SD (average), < 1 SD (small), and > 1 SD (heavy) at birth and weaning. Interaction between base and oil was not significant so percentages reported for main effects only.

[2]Displays the percentage of piglets that either stayed within the same weight category (0), increased 1 or 2 SD (1 and 2, respectively), or decreased 1 or 2 SD (-1 and -2, respectively).

[3]The percentage of pigs that died or fallbacks that were moved to the birthdeck in comparision to piglets that remained on the sow until weaning.

Table 2.8 Performance of young weaned pigs fed diets with or without Citristim and with or without a mint oil blend over a 4-phase feeding program.

[1]Items	Control	Citristim	Control	Citristim	SEM	P-value
			Mint oil+	Mint oil+		
Body weight, kg						
Wean	5.97$_{b}$	6.41$_{a}$	6.38$_{a}$	6.22$_{ab}$	0.12	0.016
d6	6.68$_{b}$	7.12$_{a}$	7.01$_{ba}$	6.75$_{ab}$	0.12	0.015
d13	8.97	9.07	9.26	9.02	0.16	0.478
d19	11.19	11.31	11.5	11.32	0.19	0.636
d35	19.78	19.8	19.17	19.96	0.35	0.303
Daily gain, kg/d[2]						
d0 – 6	0.13$_{a}$	0.12$_{ba}$	0.11$_{abc}$	0.09$_{c}$	0.01	0.01
d6 – 13	0.31	0.28	0.29	0.28	0.01	0.199
d13 – 19	0.38	0.35	0.37	0.34	0.02	0.282
d19 – 35	0.53	0.53	0.5	0.5	0.02	0.431
d0-13	0.23$_{a}$	0.2$_{ab}$	0.21$_{ab}$	0.19$_{b}$	0.008	0.012
d0-35	0.40$_{a}$	0.39$_{ab}$	0.38$_{ab}$	0.37$_{b}$	0.01	0.035
Daily feed intake, kg/d[2]						
d0 – 6	0.13	0.13	0.13	0.12	0.006	0.23
d6 – 13	0.29$_{abc}$	0.30$_{b}$	0.27$_{c}$	0.30$_{ab}$	0.008	0.002
d13 – 19	0.39$_{b}$	0.45$_{b}$	0.45$_{ab}$	0.53$_{a}$	0.02	0.0001
d19 – 35	1.08$_{a}$	1.06$_{ab}$	0.99$_{b}$	1.08$_{ab}$	0.04	0.032
d0-13	0.22$_{a}$	0.22$_{a}$	0.2$_{b}$	0.22$_{ab}$	0.005	0.026
d0-35	0.65$_{ab}$	0.66$_{ab}$	0.61$_{b}$	0.69$_{a}$	0.02	0.005
Gain:Feed[2]						
d0 – 6	0.91	0.94	0.9	0.76	0.07	0.056
d6 – 13	1.07$_{abc}$	0.91$_{cb}$	1.11$_{a}$	0.92$_{b}$	0.06	0.005
d13 – 19	0.98$_{a}$	0.79$_{ab}$	0.82$_{ab}$	0.63$_{b}$	0.06	0.0006
d19 – 35	0.5	0.5	0.51	0.46	0.024	0.448
d0-13	1.03$_{a}$	0.92$_{ab}$	1.03$_{a}$	0.88$_{b}$	0.034	0.003
d0-35	0.63$_{a}$	0.59$_{ab}$	0.62$_{a}$	0.54$_{b}$	0.022	0.018

[1]Phase 1 ran from d0 to d6 of weaning, Phase 2 was from d6 to d13, Phase 3 was from d13 to d19, and Phase 4 from d19 to d35. Pigs were weaned at 21 ± 2 d of age. [2]Calculated on a per pen basis.

Table 2.9 Distribution (%) of pigs that were deemed light and heavy at weaning throughout the post-weaning period.

	Control	Citristim	Chisq	Carrier	Mint	Chisq
			Lightweight			
Day 6						
<6.06 kg	64.0	65.0	0.980	50.0	80.9	0.072
6.06 to 7.60 kg	32.0	30.0		45.8	14.3	
≥ 7.65 kg	5.0	4.0		4.2	4.8	
Day 13						
< 8.00 kg	56.0	70.0	0.570	54.2	71.4	0.446
8.05 to 10.00 kg	25.0	40.0		41.6	23.8	
≥ 10.05 kg	4.0	5.0		4.2	4.8	
Day 19						
< 9.96 kg	64.0	64.7	0.965	60.9	68.4	0.513
9.96 to 12.50 kg	28.0	29.4		34.8	21.1	
≥ 12.55 kg	8.0	5.9		4.3	10.5	
Day 35						
< 17.05 kg	45.8	50.0	0.443	38.1	58.8	0.443
17.05 to 22.00 kg	37.5	35.7		42.9	29.4	
≥ 22.05 kg	16.7	14.3		19.0	11.8	
			Heavyweight			
Day 6						
<6.06 kg	7.0	5.7	0.014	5.0	7.9	0.922
6.06 to 7.60 kg	56.2	30.2		47.5	39.2	
≥ 7.65 kg	36.8	64.1		47.5	52.9	
Day 13						
< 8.00 kg	10.5	5.8	0.302	8.5	8.0	0.994
8.05 to 10.00 kg	42.1	32.7		37.3	38.0	
≥ 10.05 kg	47.4	61.5		54.2	54.0	
Day 19						
< 9.96 kg	10.7	5.8	0.341	10.3	6.0	0.504
9.96 to 12.50 kg	42.9	34.6		34.5	44.0	
≥ 12.55 kg	46.4	59.6		55.2	50.0	
Day 35						
< 17.05 kg	14.8	7.84	0.112	10.71	12.24	0.491
17.05 to 22.00 kg	51.85	39.22		41.07	51.02	
≥ 22.05 kg	33.33	52.94		48.21	36.73	

[1]Interaction effect was not significant; therefore, only main effects are reported

Table 2.10 Antioxidant status of sows fed diets with or without Citristim and with or without a mint top dress.

Items	Control	Citristim	Control Mint oil+	Citristim Mint oil+	SEM	P-value
SOD Serum Activity, U/ml						
D 110	7.16	10.05	10.12	5.66	4.07	0.751
Wean	4.40	4.11	5.52	5.10	0.80	0.393
Difference	2.65	5.99	4.47	0.58	3.84	0.701
SOD Colostrum and milk, U/ml						
Colostrum	73.12	65.57	75.44	76.40	10.48	0.812
D4 Milk	17.62	19.22	16.54	16.20	2.7	0.858
Difference	57.34	48.22	61.15	62.14	11.0	0.684
GSH Colostrum and milk, U/ml						
Colostrum	2.6	1.75	1.04	2.07	0.61	0.348
D4 Milk	$20.56_{x,y}$	$21.13_{x,y}$	15.16_{y}	32.05_{x}	4.93	0.059
Difference	-19.17	-19.29	-14.13	-29.76	5.09	0.112

CHAPTER 3

3.0 Reducing sow oxidative stress during gestation and lactation to improve piglet performance.

3.1 Abstract

As a means to determine nutritional strategies to alleviate the impact of oxidative stress on maternal reproductive and offspring growth performance from gestation to market, a study assessed yeast cell, mint oil, and ɣ-tocopherol supplementation into gestation and lactation diets. A total of 53 sows and gilts (206.2 ± 35.3 kg at breeding) were assigned to one of 4 diet regimens: Control (CON), control + yeast cell at 0.15% (YC), control + mint oil at 10 ppm (MO), and control + ɣ-tocopherol at 200 ppm (GT). Diets were introduced d 2 post-breed and provided in increasing amounts over a 3d adaption period. Afterwards, diet were provided from 5 d post-breed through to weaning (lactation day 21 ± 2). Control diets were formulated to meet nutrient requirements in gestation (3279 kcal ME/kg, 0.63% SID Lys) and lactation (3279 kcal ME/kg, 1.06% SID Lys). Yeast cell, mint oil and ɣ-tocopherol were added as a top dress once daily. At weaning, a total of 605 piglets (6.14 ± 2.53 kg BW) were randomly allotted to 62 pens, balanced by weight and litter within maternal diet. Pens of pigs were given a common diet for 126 d post-wean in a 9-phase feeding regimen. After d29 post-wean, performance of pigs deemed light (<5.10 kg) and heavy (>7.25 kg) at weaning were followed to d126. Sow variables evaluated were weight at beginning and end of each period, feed intake, litter characteristics at birth, and antioxidant status in serum, colostrum and milk. Offspring growth performance in the suckling and post-wean period was determined. Data were analyzed as randomized complete block and Tukey's adjustment as means

separation test. Maternal diet had minimal impact on gestation or lactation feed intake or sow BW. There was no effect on litter size or piglet weight at birth. Piglets from GT-fed sows tended to be heavier at weaning (P<0.05) than YC piglets due to differences in daily gain. The GSH content in colostrum and d14 milk samples did not differ by maternal treatment. On d14, milk GSH content was 40%, 59%, 62%, and 51% greater in CON, YC, MO, and GT sows, respectively, compared to colostrum. Colostrum concentration of SOD tended to be lower (P<0.10) in YC-fed females than MO. No difference between treatment groups for SOD content in serum and d14 milk was observed. Pigs from CON sows tended to be lighter (P<0.05) than pigs from all other treatment groups at weaning and d29 post-wean due to differences in daily gain. Lightweight MO and GT pigs were heavier at d42 (*P*=0.002) than CON and YC pigs. At d70 post-wean, pigs from GT sows tended to be heavier (P<0.10) than CON pigs, with YC and MO intermediate. Lightweight pigs from MO sows had greater gain (*P*=0.04) during the finishing period than all other treatment groups, with GT pigs gaining the least. There were no detectable differences in BW during the finishing phase among treatments in heavyweight pigs, however, CON pigs tended (*P*=0.07) to gain the least. Exposure to mint oil and ɣ-tocopherol during the prenatal and suckling period may provide lasting benefits to light-weight offspring post-weaning. Inclusion of ɣ-tocopherol in sow gestation and lactation diets enhanced suckling piglet growth; connection to sow antioxidant status remains unclear.

Key Words: Gestation, Lactation, Lightweight, Offspring, Oxidative Stress, Performance, Sows

3.2 Introduction

As previously discussed, oxidative stress is the imbalance in production of free radicals and the antioxidant defense system (Burton and Jauniaux, 2011). This state of oxidative stress can arise in different phases of an animal's life that can be defined as periods of amplified stress. Wen et al. (2019) define reproductive stress as the non-specific response of the body to reproductive activities including the estrous cycle, pregnancy, parturition and lactation. Late gestation and the lactation period are noted points where body tissue mobilization has been observed to occur. Fetal weight gain past day 69 of gestation increases rapidly (McPherson et al., 2004). As commercial feeding protocols limit feed intake in gestation, there is a potential that intake is insufficient in providing necessary nutrients for fetal growth and maintenance of maternal BW. Inadequate supply of nutrients, particularly lysine, can hinder growth of offspring and dam. In the event of insufficient nutrient supply, the sow will mobilize body tissues for nutrients to maintain fetal growth (Yang et al., 2009). While in lactation, dams are unable to consume enough feed to match milk yield (Koketsu et al., 1997; Vadmand et al., 2015) resulting in body protein mobilization in order to produce sufficient milk for offspring to grow. Mobilization of body tissue for long periods of time potentially increases the incidence of oxidative stress as nutrient breakdown could increase ROS production. Chapter 2 confirmed that lactation is associated with an increase state of oxidative stress in the female, but also illustrated supplementation of feed additives could offer support both to the dam and her offspring. Supplementation of feed additives with antioxidant capacity over the entire gestation period could result in better effectiveness in reducing oxidative damage in both gestation and lactation.

There is little research on the potential carry over effects of the inclusion of antioxidant rich feed additives in gestation and lactation diets on offspring post-weaning performance. In Chapter 2, it was observed that the inclusion of either yeast cell wall components or peppermint oil in lactation benefited offspring performance in the early post-weaning period, in particular for offspring deemed lightweight at birth and weaning. Vitamin E is also known for its antioxidant activity. Derivatives of vitamin E are also found to possess the same antioxidant activity in their chemical make-up. Gamma-tocopherol, RRR stereoisomer and natural source, is a lesser known tocopherol derivative of vitamin E. Little research has investigated the effectiveness of ɣ-tocopherol against oxidative stress. However, if similar to α-tocopherol, this source may have potential in mitigating the negative effects of oxidative stress.

The objective of this study was to evaluate the impact of separate supplementation of yeast cell wall component, peppermint oil, and ɣ-tocopherol in gestation and lactation diets on sow antioxidant status and offspring performance during the suckling and post-weaning period.

3.3 Materials and Methods

The experiment protocol was approved by the South Dakota State University Animal Care and Use Committee (17-072A) and followed the Guide for the Care and Use of Agricultural Animals in Research and Teaching (Third Ed., 2010). Animal work was conducted at the South Dakota State University Swine Education and Research Facility, Brookings, SD with the first block of weaned animals housed at the Southeast Research Farm, Beresford, SD. The study was done in two blocks, the first was conducted from November, 2018 to July, 2019 and the second from December, 2018 to August, 2019.

3.3.1 Animals and management

Twelve to fourteen animals per treatment (PIC 1050; 206.21 ± 35.26 kg) in two blocks were housed in gestation stalls (0.61 m x 1.98 m) from breeding to d110 of gestation. Sows and gilts were moved to a farrowing room and housed in individual farrowing crates from d 110 of gestation until weaning (21 ± 2d). Females were offered a common gestation and lactation diet with test ingredients added as a top dress throughout the study following a 3d adaption period at trial start. Sow feed allowance in gestation and lactation followed that described in Chapter 2, section 2.3.1. Water was provided ad libitum during both periods. Details of daily care and management, including veterinary treatments during gestation, farrowing, and the suckling period were consistent with those illustrated in section 2.3.1.

Due to limited finishing space, pigs from block 1 were weaned to the Southeast Research Farm, Beresford, SD and pigs from block 2 were weaned to the Wean to Finish barn located on the South Dakota State University Swine Education and Research Facility, Brookings, SD. In the first weeks after weaning, pens were over-stocked at 13 to 14 pigs/pen in block 1 and 7 to 9 pigs/pen in block 2 within maternal treatment (605 pigs total at 6.14 ± 2.53 kg; 11-19 pens/maternal treatment); balanced for weight and litter within pen as possible. Pen stocking density at both facilities are based on finishing pig space requirements and thus initial pigs/pen was unlikely to influence pig performance up to 7 weeks after weaning (Johnston et al., 2017). Equisul (400 mg/mL, 180 mL/gal water) and liquid aspirin were given through water medicators for at least one week starting at weaning with an additional week if looseness was still apparent. Feed and water were offered ad libitum. Pen stocking density was reduced to 5 and 6 pigs/pen at d29 and 42

after weaning in block 1 and 2, respectively. From both blocks, pigs that were sold were those that were denoted as average at weaning with the retained being light and heavy animals. Details of veterinary treatments during the post-wean period are consistent with those illustrated in section 2.3.1. All pigs and facilities were checked once daily by a trained research unit manager; and with the assigned graduate research assistant checking pigs at the onsite wean to finish barn three times a week during the course of the study.

2.3.2 Experimental design and dietary treatments

Sows and gilts were randomly assigned to one of 4 experimental diets: standard diet with carrier (Control), standard diet with Citristim (Citristim), standard diet with mint oil (MO), and standard diet with ɣ-tocopherol (GT). All four dietary groups were top dressed with their respective test ingredient once daily at 50 g/d (set to ensure 0.15% for Citristim; 10 ppm active ingredient for mint oil; and 200 ppm for ɣ-tocopherol) at the 0600h feeding during gestation and 0800h feeding during lactation. An adaption period of dietary treatments was implemented within 2d of breeding, with the experimental period occurring at d5 of breeding and maintained through weaning to reduce refusals. Gestation and lactation diets were formulated to meet or exceed nutrient requirements for sows according to NRC (2012). Feed orts were removed and weighed every 3 d for determination of sow feed disappearance.

Pigs were fed a common diet throughout the post-wean period. All diets were formulated to meet nutrient requirements of weaned pigs within a 9-phase feeding program: Phase 1, 5d; Phase 2, 4-6d; Phase 3, 7-13d; Phase 4, 10-23d; Phase 5, 19-25d; Phase 6, 16-23d; Phase 7, 21-24d; Phase 8, 12-20d; and Phase 9, 4-10d. The trial ended at d126 post-wean for both blocks. Phase 1 and 2 were provided in pellet form (Ralco

Nutrition, Marshall, MN) with all following phases provided as a meal; Paylean (Elanco,) was added to Phase 9. Water was provided ad libitum.

3.3.3 Data collections, chemical analyses and calculations

Sow BW was measured at breeding, d110 of gestation, within 24 hours of parturition, and at weaning; back fat measured at breeding, d110 of gestation and at weaning. In concert with BF, blood samples were collected via jugular venipuncture into a nonheparinized blood collection tube (BD Vacutainer, Franklin Lakes, NJ) and a heparinized blood collection tube (BD Vacutainer, Franklin Lakes, NJ). Samples were kept on ice at collection and then stored at -80°C for later analysis of antioxidant activity. Litter characteristics (total born, born alive, stillborn, mummies, gender distribution) were recorded. Collection methods for colostrum, d4 and d14 milk samples are detailed in section 2.3.3. Colostrum and milk samples were analyzed for protein, lactose, total solids, and fat at the Division of Regulatory Services, (University of Kentucky, Lexington, KY). Fecal samples were collected at trial start and d110 of gestation in block 2 from 5 sows per treatment group. A rice size of fecal matter was collected with a DNA kit (Biopolis, Valencia, ES) with a 1g sample transferred to a 15 ml conical tube with 5ml of PBS with glycerol (# G5516 Sigma-Aldrich, St. Louis, MO). In concert, a 2ml subsample of colostrum and milk were stored in a 5ml microcentrifuge tube with glycerol. Fecal, colostrum, and milk samples were analyzed for microbiome and *Caenorhabditis elegans* analysis at Biopolis. Microbial analysis of maternal fecal samples at d110 of gestation was also conducted to determine the proportion of *Lactobacillus* and *Bifidobacterium*. Isolation of fecal microbial DNA occurred with the use of DNeasy PowerSoil Kit (MO BIO Laboratories, Qiagen, Venlo, Netherlands).

Following the completion of the trial, subsequent breeding and farrowing characteristics were evaluated.

Suckling piglets were weighed within 24 hours of farrowing, at d4 and d7 of lactation, and at weaning. Similar to the previous study, BW of the population across both blocks was compiled and used to establish three weight categories at each weigh day. Determination of weight groups are detailed in section 2.3.3. Category change for each weigh period was calculated to determine the percentage of animals that maintained, moved up or fell back a weight category. Veterinary treatments for scours during the suckling period were recorded on an individual pig basis.

Time required for newly weaned piglets to eat was assessed during the first four days after weaning. Biscuits containing ferric oxide were crumbled up to match sizing of Phase 1 pelleted feed and added at 5% of the total amount allotted per pen. Fecal color change of each pig was measured at d2 to 4 via rectal swab. Digestion of marker would not color feces until 24 to 36 hours after consumption, thus identification of color change would then indicate a possible time when pigs ate.

After weaning pigs were weighed at the end of each diet phase until d42, then monthly until trial completion. Feed disappearance was recorded in tandem with weigh days. ADG, ADFI, and G:F was calculated to determine pen performance. Veterinary treatments of individual animals were recorded to assess overall health status of treatment groups. As in the suckling stage, piglets were categorized as light, average, and heavy at each weigh period. Change in category assignment (i.e. fell back, went up, or maintained) over time was monitored on an individual basis. Analysis of weight categories were done

up to d29 post-wean due to removal of pigs for the purpose of stocking density as described previously.

A pooled feed sample was collected for each batch of feed delivered through the study and analyzed as described in section 2.3.3.

On d2 of lactation, a 1 mL blood sample was collected from the mammary vein of three piglets from each litter (one piglet/birth weight category). Samples were centrifuged at 28,000 x g for 20 minutes. Avoiding the blood clot at the bottom of the vial, serum was stored in a microcentrifuge tube at -80°C until time of analysis. Immunocrit analysis was based on the methods of Vallet et al. (2013). Serum was diluted in a 1:1 ratio with 40% ammonium sulfate in distilled water. The newly diluted sample was loaded into a microcapillary tube and placed into a hematocrit centrifuge (MX12 PCV Centrifuge, LW Scientific, Lawrenceville, GA) and was centrifuged at 12,000 x g for 10 minutes. The length of Ig precipitate and the length of diluted colostrum was measured and divided to determine the immunocrit ratio (IR). Ideally, three pigs were chosen from each litter but if no pigs in the litter fit a certain weight range, the observation for that category was then lost. In conjunction with piglet sera samples, immunocrit was evaluated in colostrum. A modified methodology from Vallet and Miles (2017) was used. Colostrum samples were diluted in a 1:1 ratio with 10% bovine serum albumin (1ml: 9ml Saline; Fisher BP6751) in 0.9% saline. In duplicate, diluted colostrum samples were combined with 40% (wt/vol) ammonium sulfate in distilled water to precipitate immunoglobulins and then loaded into a hematocrit centrifuge and centrifuged at 12,000 x g for 10 minutes. Immunocrit ratio was determined as the ratio of the precipitate length divided by the total length of diluted colostrum, then doubled to account for prior colostrum dilution.

Serum insulin-like growth factor 1 was measured in serum from d110 of gestation as well as colostrum and milk samples. IGF-I concentration was determined in duplicate by radioimmunoassay (Echternkamp et al., 1990; Funston et al., 1995) for blood, colostrum, and milk samples. Insulin-like growth factor binding proteins (IGFBP) were extracted from serum using a 1:17 ratio of sample to acidified ethanol (12.5% 2 N HCl: 87.5% absolute ethanol; Daughaday et al., 1980). Extracted samples were centrifuged (12,000 × g at 4°C) to separate IGFBP. A portion of the resulting supernatant was removed and neutralized with 0.855 M Tris base, incubated for an additional 4 h at 4°C, and then centrifuged at 12,000 × g at 4°C to remove any additional IGFBP. When samples of this extract, equivalent to the original serum sample, were subjected to Western ligand blot analysis and subsequent phosphorimagery, no detected binding of 125I-IGF-I to IGFBP was observed. Inhibition curves of the neutralized extracted serum ranging from 12.5 to 50 µL were parallel to the standard curve. Recombinant human IGF-I (GF-050; Austral Biological, San Ramon, CA, USA) was used as the standard and radioiodinated antigen. Antisera AFP 4892898 (National Hormone and Peptide Program, National Institutes of Diabetes, Digestive and Kidney Diseases, Bethesda, MD, USA) was used at a dilution of 1:62,500. Sensitivity of the milk and colostrum assay was 6.01 pg/tube and intra-assay CV was 3.9%. Sensitivity of the assay for measuring serum concentrations of IGF was 14.7 pg/tube. Intra- and interassay CV were 8.6% and 7.3%, respectively. Correlation between milk/colostrum and sow serum circulating IGF-1 was assessed.

Superoxide Dismutase, glutathione, and glutathione peroxidase concentration in serum and milk were measured as a means to determine oxidative status of the female.

SOD enzyme concentration was determined by a commercially available kit as detailed in section 2.3.3. GSH was assessed with the use of a Glutathione Assay Kit (Cayman Chemical, Ann Arbor, MI). Samples were deproteinated in accordance to manufacturer's instructions, which is illustrated in section 2.3.3. Plasma concentration of GSH was unable to be analyzed due to an inability to locate a lab that could conduct the analysis.

2.3.4 Statistical analysis

Variables of particular interest include sow reproductive performance (i.e. litter size, lactation feed intake), sow antioxidant status and piglet performance (i.e. piglet BW, post-wean feed intake, growth rate during lactation and in the nursery). Data was analyzed using the mixed model procedure of SAS (Version 9.4, SAS Inst. Inc., Cary, NC) considering the effect of dietary supplementation where the sow is the experimental unit and sow (block) is the random effect and with pen as the experimental unit and pen (block) as the random effect for post-wean. Due to different housing locations in the post-weaning period, location was included in the model for post-weaning performance. Tukey's adjustment was used for means separation test. Significant differences were reported at $P < 0.05$ and tendencies for significance were reported when $0.05 \leq P \leq 0.10$.

Weight categories, category change, and fecal color evaluation were analyzed using the Freq procedure in SAS (Version 9.4, SAS Inst. Inc., Cary, NC).

3.4 Results

Of the 53 females that were on placed on treatment, 52 successfully completed the trial. One sow was euthanized during farrowing due to complications of a twisted uterus. Data collected from this female prior to farrowing were included in the results.

3.4.1 Diet Analysis

Tables 3.1 and 3.2 report AA and proximate analysis of gestation, and lactation and Phase 1 to 9 diets, respectively. For gestation, CP content differences of the seven batches ranged from 0.20% to 3.24%. Lysine, threonine, tryptophan, methionine, cystine, isoleucine and valine content in the gestation batches differed by 0.03% to 3.00%. The three lactation batches had a CP that differed by 0.37 to 0.53%. As for AA content, percentages of each amino acid differed by less than 1%.

3.4.2 Farrowing and Suckling Growth Performance

There was no effect of maternal diet on sow BW or backfat throughout the trial (Table 3.3). Sows in GT group tended to have greater (P<0.10) feed intake in gestation than Citristim. There was no difference in sow lactation feed intake or litter characteristics at birth (Table 3.3). Incidence of scours was low, as evidenced by number of pigs/litter treated for scours. Pigs removed (died or fall back) was within industry standards and 4 – 7% lower in MO and GT litters. Piglet BW was not different at birth; considering all pigs weaned, GT pigs tended to be heavier (P<0.10) at weaning. The pattern was similar when considering only suckled piglets and reflects slight differences in ADG. Considering distribution of piglets in light, average, and heavy weight categories in the suckling period, in all groups the proportion of light weight piglets increased at d4 from birth (Table 3.5). However, at the end of weaning, the proportion of lightweight animals from d4 in the MO and GT groups, decreased primarily due to a tendency for a greater number of piglets maintaining BW or increasing by at least 1 SD (Table 3.5). There was no difference in d2 immunocrit value (Table 3.6) by sow treatment or birth weight category. Similarly, colostrum immunocrit was not affected by maternal dietary treatment. There was a tendency for a main effect of maternal treatment

on serum concentration of IGF-1 in d4 milk but no differences between treatments were noted based on Tukey's adjustment (Table 3.6). IGF-1 in colostrum was almost 2-fold higher than in milk.

3.4.3 Post-wean Growth Performance

The proportion of colored feces within 24h after weaning in Citristim, MO, and GT pigs were 2-fold greater than that of the Control (10 – 12% vs 4.7% of Control pigs; Table 3.8). Pigs from Citristim sows were heavier ($P<0.05$) with pigs from MO and GT sows tended to be heavier ($P< 0.10$) at d29 after weaning than pigs from Control sows (Table 3.7) which is in part due to greater ($P<0.05$) feed intake over the same period and hence, daily gain. An increasing proportion of pigs falling back after weaning were observed for pigs from Control-fed (Table 3.8).

Veterinary treatments administered in throughout the post-wean period was recorded in both barns. No treatments were given to the pigs housed at the offsite facility, thus values reported in table 3.8 encompass onsite records. Percentage of animals treated were similar between treatment groups, with GT treating a slightly lesser amount.

At on-site, lightweight pigs from MO and GT sows were heavier ($P<0.05$) at d42 and tended to be heavier ($P<0.10$) up to d126 (Table 3.9). No difference in BW was observed for heavyweight pigs throughout the finishing period; although MO pigs were around 3 kg heavier than all other treatment groups at d126. Lightweights from the MO group had higher gain ($P<0.05$) than all other treatments throughout the finishing period, while pigs from GT sows tended to have lesser gain. Heavyweight pigs from Control group tended to gain less ($P<0.10$) than the MO or GT groups from d98 up to d126. At off-site, lightweight pigs from Citristim sows were lightest at d42 and tended to remain

lighter through to d126 (Table 3.10). There was no detectable difference in BW of heavyweight pigs by maternal treatment; however, pigs from Citristim, MO, and GT groups were approximately 3, 8 and 6 kg heavier at d126 than pigs from Control sows. These differences in BW are in part reflective of differences in daily gain. As for veterinary treatments, there was a larger number of pigs treated from Citristim-fed sows than all other treatments (Table 3.7).

3.4.4 Antioxidant Activity

Colostrum and milk GSH content was not affected by maternal treatment with a 4% greater concentration in milk than colostrum (Table 3.11). Considering sow parity, colostrum GSH tended to be greater ($P<0.05$) in gilts than multiparous females (Table 3.12); parity did not have an effect on GSH concentration in d4 and 14 milk. Superoxide Dismutase content in sow serum was not affected by maternal treatment. There appeared to be an effect of time on sow serum SOD content where concentration was 2-fold greater at d110 relative to breeding and weaning. Parity did not have an effect on SOD content in serum (Table 3.12).

There was no difference in proportion of *Lactobacillus* and *Bifidobacterium* in sow feces at d110 of gestation (Table 3.4). However, it is of note a slightly greater proportion of *Lactobacillus* and *Bifidobacterium* was reported in the MO and GT groups.

3.4.5 Subsequent Performance

In the subsequent parity, return to estrus was similar across all the treatment groups at around 4.3 days. From this study, 12 to 14 sows were rebred, with only 11 from CON, MO, and GT and 9 from the YC group farrow in a subsequent litter. As well, GT

and CON females had an average born alive of around 14.5 to 15 pigs with YC and MO averaging 14.

3.5 Discussion

The objective of the study was to assess the impact of Citristim, peppermint oil, and ɣ-tocopherol supplementation on sow antioxidant status during gestation and lactation and its potential carryover effects on offspring performance from birth to d126 post-wean. In relation to sow performance, there were minor effects of feed additive inclusion on sow performance, including weight changes in gestation and lactation, and litter characteristics at birth. The greater gestation feed intake of the Citristim group is more likely related to initial sow allocation than treatment where 1, 0, 2, and 4 sows in Control, Citristim, Mint oil, and ɣ-tocopherol groups had a BCS of 1. As such, daily feed allocation was greater to achieve target body condition. Minor impacts on sow performance with the addition of phytochemicals or yeast cell products have similarly been reported by Bass et al (2019), Meng et al. (2018), and Tan et al. (2015).

With respect to offspring performance, BW of piglets during the suckling period did not differ until weaning, with all animals from ɣ-tocopherol being heaviest. This difference in average wean weight was not observed, however, in other phytochemical supplementation studies (Tan et al., 2015; Meng et al., 2018; Lipiński et al., 2019). Table 3.3 displays the number of deads out of each group, as well as percent removed. All but one of the treatment groups had about 30 piglets die during the suckling period, with the mint oil group having 20. However, with respect to those removed, both mint oil and ɣ-tocopherol had a lower percentage removed indicating that they appear to provide benefit in reducing the number of fall backs. Maternal dietary treatment did not affect the

percentage of pigs testing positive for fecal color change in the early post-wean period. Interestingly, the proportion of pigs that were positive at d2 compared to Control was larger in all three treatment groups. The low proportion of red-colored feces detected in this group implies that, while pigs had likely consumed some feed, most were not on full feed by day 2. An aversion to the biscuit causing them to eat around it may also have contributed to the low red-colored feces. Pigs from the treatment groups had a better feed intake in first days after weaning likely also contributed to the heavier weights at d4 post-wean. As Control pigs had a slower transition to dry feed in first few days after weaning explains why they tended to be lighter at d29 post-wean.

Assessment of scour treatments per litter in the suckling period was done to estimate health status of the herd. Citristim, which is derived from *Pichia guilliermondii*, possesses the ability to adhere to pathogens found in the intestinal lumen of animals. Health status was also evaluated during the post-wean period of the study. Interestingly, the percentage of animals treated were numerically higher in the Citristim group than all other treatments. Yeast products offer additional support to the animal as they are immunomodulators. Inclusion of yeast strains in diets may only be effective during the time of addition as they bind to pathogens and are excreted with the bound toxin. If Citristim was added to the diets post-wean, it could be possible that the number of animals treated would have been reduced. Antioxidants provided by the inclusion of peppermint oil and ɣ-tocopherol in gestation and lactation diets possibly carried over in the post-wean period. As the antioxidants are potentially still present in the offspring's body, combined with maturation of the defense system, it is likely that the incidence of sickness was reduced.

Antioxidant activity was not affected by dietary treatment. It was observed that colostrum SOD activity was lower for females fed Citristim compared to mint oil, however, it is unsure at this time for why this was found. A higher concentration of SOD in the sample indicates that the animal may be under oxidative stress as there is greater need of the SOD enzyme to dismutase 50% of the superoxide anion present. If the concentration of GSH measured in the sample was low, it could indicate that the animal was under a state of oxidative stress as well as there is a lower amount of the antioxidant present to detoxify ROS. As Citristim is a yeast-based product, it is not known to provide antioxidant activity and more known for pathogen binding. Other phytochemical studies detected an effect on oxidative state in sows. Meng et al. (2018) observed that SOD activity in milk from the Resveratrol-fed sows was higher than Control sows at d 0 and 14 of lactation but did not differ at d 7 and 21 of lactation. The concentration of SOD in colostrum and milk decreased as lactation progressed, while the concentration of GSH increased. This pattern of increasing oxidative status with progression of lactation was similarly noted in the previous trial. Gilts and sows past their fifth parity are found to possess a lower antioxidant capacity (Lipko-Przybylska and Kankofer, 2012). Lower capacity may indicate that these females are less successful in reducing free radical concentration compared to their older counterparts. Thus, they may be likely to experience oxidative stress more frequently or undergo it at a faster rate. Although not different, the concentration of SOD appeared to be higher in gilts during lactation. Which may indicate that intervention of oxidative stress may only need to occur in specific parities as oppose to all parities. It was suggested by Lipko-Przybylska and Kankofer

(2012) that it may be best to supplement sows before their first ever farrowing and those that have had five litters or more.

Inclusion of antioxidant rich feed additives during gestation and lactation would offer aid to the dams against stress, however, it could also potentially support her offspring. Pre-and postnatal exposure to antioxidants may aid piglets later in life. During the first trimester, antioxidants are found to be transferred into the exocoelomic cavity and subsequently into the fetal gut and circulation via the secondary yolk sac in humans (Jauniaux et al., 2004). It is unknown if this maternal-offspring transfer is found in the porcine species. Homo sapiens are characterized with a hemochorial placenta, whereas swine have an epitheliochorial placenta. Although there is some structural difference of the two placental types, it can be argued that the mean difference is the rate and type of passage of nutrients from mother to fetus. For swine, the placenta has a six layered epitheliochorial structure and is impermeable to the passage of macromolecules such as immunoglobulins (Bode et al., 2010). Although the placenta is impermeable to macromolecules, it is likely antioxidant size is small enough to pass through. It has been found that resveratrol, polyphenol from the stilbene family of phytoalexins, was capable of crossing the placenta and affecting the fetus directly in both swine and rats (Bourque et al., 2012; Meng et al., 2018).

The immunocrit values at d2 did not differ by weight category or treatment, suggesting differences in piglet weight at weaning may be related to in-utero fetal development and/or milk nutrient supply. It is vital that piglets consume enough colostrum in the first two days of life to obtain enough energy, nutrients, and immune support to help with their growth. If they are poor eaters, it could lead to lower BW and

increased mortality rate. However, piglets out of the lightweight category possessed an IR that was numerically higher to their heavier weighted littermates. Possibilities as for why this occurred could be due to two cases. For this study, piglets were weighed within 24 hours after parturition, thus birth order was not recorded. Piglets born in the first half of farrowing may have higher immunocrit values as they would have more time at the teats with less competition. If birth order was recorded, it would have been interesting to see if pigs that were selected to be bled fell within the first or second half. The second item that could have affected the ratio is split suckling. Allowing lighter littermates to suckle without competition can result in colostrum intake to increase, potentially decreasing the risk of pre-wean mortality. Vallet et al. (2013) reported that a ratio at or above 0.125 coincided with a higher survivability rate in the suckling period. Peters et al. (2016) however, utilizing similar methods, did not find a correlation between lower IR and pre-weaning mortality.

No effect of maternal dietary treatment was noted for IGF-1 in d110 serum, colostrum, d4 and d14 milk samples. A pattern was denoted that as the lactation period progressed, the concentration of insulin-like growth factor 1 decreased within milk. Donovan et al. (1994) found that the concentration of roaming IGF-1 in colostrum for swine is 4 to 17 nM with mature milk ranging from 1 to 3 nM. The concentration of IGF-1 in colostrum and milk determined in the study is much higher than the values found in literature. However, it still follows that the concentration is higher in colostrum than mature milk. The concentration of *Lactobacillus* and *Bifidobacterium* at d110 of gestation was not affected by maternal dietary treatment. Exposure to maternal fecal matter is one way piglets are able to obtain and establish intestinal microbiota. Yusof et al. (2000)

reports that certain strains of *Bifidobacterium* have antagonistic effects against *E. coli* K88 and *Salmonella choleraesuis*. *Lactobacillus* is also effective against pathogens such as *E. coli* (Yusof et al., 2000; Gopal et al., 2001). Although the degree of variation within and between groups for fecal microbial analysis from block 2 limits conclusive interpretations, offspring exposure to slightly higher levels of these beneficial bacteria in sow feces may have also contributed to improved growth in the respective piglets.

3.6 Conclusion

Inclusion of feed additives in both gestation and lactation diets potentially improved the oxidative status of the dam, particularly Citristim. Peppermint oil enhanced performance of light weight animals during the pre and post-wean period, as previously observed in Chapter 2. The addition of ɣ-tocopherol to the sow's diet also resulted in improved growth of offspring, particularly lightweight animals. From this study, it appears that pre- and post-natal exposure to additives rich in antioxidants potentially prepares offspring to better defend against future stressors that could impair performance.

Table 3.1 Amino acid and proximate analysis of gestation and lactation experimental base diet.

	Gestation							Lactation		
Collection Time	November	December	December - January	January	February	February	March	B1: March	B2: April	B2: March
Batch No.	One	Two	Three	Four	Five	Six	Seven	One	One	Two
Items, %										
Proximate analysis										
CP	12.13	14.97	12.33	12.59	12.78	11.73	12.28	17.91	18.28	18.81
Moisture	13.40	11.56	13.32	15.34	12.11	13.71	13.36	15.63	12.59	11.60
Crude Fat	1.81	2.14	1.84	1.28	1.87	1.66	1.85	1.19	2.15	1.83
Crude Fiber	1.79	1.88	1.95	1.98	1.55	2.09	1.70	2.41	2.63	2.17
Ash	4.95	5.41	4.60	4.68	4.76	4.92	5.04	4.75	5.66	5.98
Amino Acid analysis										
Tau	1.11	1.43	1.11	1.19	1.14	1.08	1.17	1.91	1.85	1.89
Hyp	0.45	0.64	0.46	0.50	0.44	0.43	0.46	0.73	0.69	0.70
Asp	0.52	0.63	0.52	0.58	0.51	0.48	0.51	0.80	0.77	0.78
Thr	2.11	2.60	2.12	2.32	2.23	2.04	2.18	3.22	3.18	3.27
Ser	0.80	0.92	0.79	0.87	0.85	0.77	0.81	1.09	1.07	1.10
Glu	0.49	0.61	0.49	0.53	0.48	0.49	0.51	0.80	0.77	0.75
Pro	0.68	0.80	0.68	0.75	0.72	0.65	0.68	0.92	0.90	0.91
Gly	0.22	0.24	0.22	0.25	0.22	0.23	0.24	0.33	0.31	0.30
Ala	0.57	0.70	0.57	0.63	0.61	0.58	0.62	0.95	0.89	0.91
Cys	0.18	0.24	0.18	0.22	0.18	0.19	0.18	0.26	0.24	0.25
Val	0.51	0.64	0.50	0.55	0.54	0.51	0.55	0.88	0.82	0.85
Met	1.16	1.36	1.16	1.29	1.26	1.11	1.17	1.62	1.53	1.61
Ile	0.35	0.44	0.35	0.41	0.37	0.36	0.38	0.62	0.60	0.63
Leu	0.61	0.75	0.61	0.67	0.65	0.59	0.64	0.98	0.92	0.96
Tyr	0.03	0.03	0.03	0.02	0.03	0.02	0.03	0.02	0.04	0.03
Phe	0.01	0.02	0.01	0.02	0.01	0.02	0.01	0.04	0.01	0.02
Hyl	0.60	0.90	0.61	0.62	0.60	0.57	0.66	1.02	1.05	1.08
Orn	0.32	0.39	0.32	0.35	0.33	0.32	0.34	0.50	0.49	0.49
Lys	0.67	0.88	0.69	0.74	0.70	0.67	0.74	1.20	1.19	1.22

His	0.14	0.16	0.13	0.13	0.14	0.12	0.13	0.23	0.25	0.26

Table 3. 2 Amino acid and proximate analysis of the base diet of a nine-phase feeding program.

Phase	**One**	**Two**	**Three**	**Four**	**Five**	**Six**	**Seven**	**Eight**	**Nine**
Items, %									
Proximate analysis									
CP	20.07	20.67	20.24	19.80	17.09	14.02	12.00	10.86	12.89
Moisture	6.69	7.43	10.59	12.23	12.76	12.98	13.60	12.69	13.11
Crude Fat	6.35	6.54	3.31	1.39	1.86	2.05	1.89	2.09	2.25
Crude Fiber	1.94	2.03	2.00	2.02	2.76	1.94	1.64	1.48	1.99
Ash	6.57	6.29	6.00	5.07	3.98	4.28	3.76	3.44	3.48
Amino Acid analysis									
Tau	1.83	1.88	1.97	1.86	1.73	1.31	1.03	0.97	1.18
Hyp	1.02	1.01	0.87	0.81	0.77	0.61	0.53	0.48	0.66
Asp	0.82	0.82	0.84	0.79	0.77	0.59	0.49	0.48	0.56
Thr	3.22	3.24	3.41	3.31	3.11	2.40	2.01	1.96	2.24
Ser	1.01	1.02	1.10	1.06	1.04	0.89	0.80	0.79	0.84
Glu	0.94	0.93	0.84	0.74	0.73	0.56	0.46	0.44	0.52
Pro	1.03	1.04	0.98	0.92	0.90	0.74	0.66	0.65	0.71
Gly	0.30	0.29	0.30	0.33	0.33	0.27	0.21	0.20	0.24
Ala	1.01	1.03	0.94	0.90	0.85	0.66	0.55	0.52	0.60
Cys	0.53	0.51	0.42	0.51	0.36	0.28	0.22	0.18	0.30
Val	0.86	0.87	0.88	0.83	0.77	0.59	0.48	0.45	0.53
Met	1.58	1.61	1.65	1.62	1.52	1.25	1.10	1.10	1.21
Ile	0.69	0.68	0.66	0.66	0.61	0.45	0.36	0.35	0.37
Leu	0.94	0.94	0.95	0.94	0.88	0.69	0.58	0.55	0.64
Tyr	0.03	0.03	0.04	0.03	0.03	0.03	0.03	0.03	0.03
Phe	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Hyl	1.47	1.58	1.40	1.29	1.20	1.02	0.92	0.84	0.93
Orn	0.45	0.46	0.50	0.48	0.46	0.37	0.31	0.30	0.35
Lys	1.19	1.20	1.21	1.18	1.11	0.83	0.66	0.62	0.74
His	0.30	0.30	0.26	0.21	0.20	0.16	0.13	0.11	0.16

Table 3.3 Performance of sows fed one of four treatments throughout gestation and lactation.

Items	Control	Citristim	Mint Oil	γ-tocopherol	SEM	P-value
Sow per treatment	13	14[1]	12	14		
Parity						
No. P0	3	3	1	3		
No. P1	1	1	3	1		
No. P2	2	2	1	3		
No. P3	3	2	2	1		
No. P4	2	4	1	5		
No. P5	2	2	4	1		
No. Savaged	3	3	1	3		
Sow BW, kg						
Breeding	203	190	214	208	12.80	0.583
d 110	238	223	250	241	11.48	0.377
Farrowing[2]	231	236	234	229	7.60	0.881
Weaning	224	228	233	228	7.05	0.832
Change, Breeding to d110	37.5	36.6	37.9	34.8	5.65	0.969
Change, d110 to Wean	-1.9	-2.8	-1.4	-1.1	5.56	0.995
Sow back fat, mm						
Breeding	17.4	17.3	16.8	17.6	0.61	0.797
d 110	19.0	20.3	18.4	20.0	1.01	0.394
Wean	16.8	19.1	16.6	18.0	1.38	0.357
Change, Breeding to d110	2.0	2.4	2.0	2.8	0.81	0.866
Change, d110 to Wean	-2.2	0.4	-1.8	-2.0	2.04	0.701
Feed intake, kg/d						
Gestation	2.07[x,y]	2.03[y]	2.22[x,y]	2.22[x]	0.06	0.029
Lactation	6.50	6.71	6.95	6.80	0.23	0.558
D4 Milk Composition, %						
Fat	7.89	7.05	7.77	7.45	0.55	0.600
Protein	5.01	5.06	5.12	4.77	0.15	0.264
Lactose	5.34	5.39	5.32	5.23	0.14	0.815
Total Solids	19.49	18.69	19.47	18.67	0.61	0.529
Solids not Fat	10.62	10.74	10.74	10.34	0.16	0.186
D14 Milk Composition, %						
Fat	7.37	8.07	6.78	7.13	0.50	0.296
Protein	4.82	4.63	4.75	4.48	0.13	0.159
Lactose	5.76	5.77	5.77	5.93	0.12	0.454
Total Solids	19.06	21.44	18.30	18.59	0.98	0.111
Solids not Fat	10.70	10.56	10.60	10.53	0.14	0.729
Litter, average						
Born Alive	15.0	13.8	14.8	14.4	0.93	0.855
Stillborn	1.00	1.00	1.60	1.10	0.54	0.794
Mummies	0.61	0.58	0.42	1.00	0.33	0.616
Weaned	12.8	12.2	12.5	12.4	0.59	0.927
Scour Treatment[3]	2.9	4.5	1.3	0.9	1.26	0.146
Piglets, number						
Born alive	198	188	180	200		
Stillborn	17	16	22	19		

Mummies.	8	8	5	14		
Weaned	165	157	160	167		
Pulled Off and Lived	11	10	8	2		
Deads[4]	33	31	20	33		
% removed	22.2	21.8	15.6	17.5		
Piglet BW[5], kg						
Birth	1.34	1.34	1.35	1.36	0.03	0.952
d4 of lactation	1.76	1.85	1.83	1.83	0.04	0.253
d7 of lactation	2.39	2.39	2.43	2.41	0.06	0.945
Wean, all	$5.41_{x,y}$	5.34_{y}	$5.63_{x,y}$	5.77_{x}	0.14	0.040
Wean, suckling	5.55	5.66	5.69	5.90	0.14	0.313
Lbs weaned/sow, suckling	152	145	158	156	10.90	0.812
Piglet ADG, kg/d						
d 0-4	0.08	0.09	0.09	0.10	0.01	0.711
d 4-7	0.15	0.23	0.11	0.14	0.05	0.274
Wean, all	0.20_{b}	$0.21_{b,x}$	0.22_{ab}	$0.23_{a,y}$	0.01	0.011
Wean, suckling	0.20	0.21	0.21	0.22	0.01	0.262

[1]One sow was euthanized during farrowing due to a twisted uterus.
[2]Females were weighed within 24 hours of parturition.
[3]Presented values are the average number of individual treatments/litter.
[4]Includes pigs that died and fall backs/starveout who were moved to birth decks that did not make it to weaning.
[5]Data represents pigs that were kept on the sow from farrowing to weaning unless otherwise denoted.

Table 3.4. Relative proportion (ΔΔCt) of Lactobacillus and Bifidobacterium in sow fecal samples at d110 of gestation.

Items	Control	Citristim	Mint Oil	γ-tocopherol	SEM	P-value
Lactobacillus	8.41	7.78	9.34	8.22	1.49	0.835
Bifidobacterium	1.22	1.57	0.48	2.62	1.42	0.717

[1]Relative proportion was determined as ΔΔCt = [(CtLactobacillus from normalizing sample) – (CtLactobacillus from samplei) – CtUniversal from normalizing sample) – CtUniversal from samplei. Normalizing sample for each target genus was defined as the sample with the highest Ct value.

Table 3.5 Distribution of piglets within litter in weight categories[1].

	Control	Citristim	Mint Oil	γ-tocopherol	Chisq
n	148	156	149	152	
Birth weight range, kg					
<1.1	16.0	13.6	20.6	19.5	0.569
1.15-1.65	60.9	64.0	58.2	54.4	
1.66-2.6	23.1	22.5	21.2	26.0	
Day 4 lactation range, kg					
0.8-1.55	26.6	24.5	29.0	27.5	0.127
1.6-2.1	57.8	50.3	44.8	44.9	
2.15-3.5	15.6	25.2	26.2	27.5	
Wean weight range, kg					
1.4-4.65	27.6	25.9	19.2	16.6	0.235
4.7-6.65	48.1	48.3	54.8	57.4	
6.7-10	24.4	25.9	26.0	26.0	
Birth to d4 Change[2]					
-2	0.0	0.0	0.0	0.6	0.183
-1	24.0	17.7	12.4	13.8	
0	70.1	72.8	77.9	77.3	
1	5.8	9.5	9.7	8.4	
2	0.0	0.0	0.0	0.0	
Change to wean[2]					
-2	2.6	0.7	0.0	1.8	0.093
-1	26.9	27.2	16.4	17.2	
0	50.0	52.4	61.6	57.4	
1	19.2	19.7	21.2	23.7	
2	1.3	0.0	0.7	0.0	

[1]Weight categories based on mean ± 1 SD (average), < 1 SD (small), and > 1 SD (heavy) at each weigh period.

[2]Percentage of piglets that stayed within the same weight category (0), increased 1 or 2 SD (1 and 2, respectively), or decreased 1 or 2 SD (-1 and -2, respectively).

Table 3. 6 Sow immune composition and piglet immunocrit at d2 of age.

	Control	Citristim	Mint Oil	γ-tocopherol	SEM	P-value
Colostrum Immunocrit	0.37	0.40	0.36	0.39	0.04	0.896
Piglet Immunocrit[1]						
Light (< 1.1 kg)	0.185	0.143	0.165	0.174	0.03	0.775
Average (1.15 – 1.65 kg)	0.154	0.179	0.159	0.189		
Heavy (> 1.65 kg)	0.174	0.167	0.180	0.209		
IGF-1, ng/mL						
Serum, d110	52.89	56.85	58.01	59.89	4.63	0.624
Colostrum	864.54	875.99	882.57	934.63	46.61	0.723
Milk, d4	298.77	306.23	303.28	299.41	3.00	0.078
Milk, d14	298.17	302.65	301.85	298.17	4.17	0.666

[1]Immunocrit values were analyzed based on weight category at birth.

Table 3.7 Performance of nursery pigs up to d29 after weaning[1].

Items	Control	Citristim	Mint Oil	γ-tocopherol	SEM	P-value
Body weight, kg						
d4	5.30	5.50	5.39	5.27	0.36	0.916
d14	7.51	8.10	7.89	8.19	0.32	0.176
d29	12.37$_{b,y}$	13.83$_{a,x}$	13.85$_{x}$	13.65$_{x}$	0.54	0.031
Daily gain, kg/d						
d0 – 4	0.04	0.06	0.01	0.05	0.02	0.118
d4 – 14	0.21$_{b,y}$	0.24$_{a,x}$	0.24$_{x}$	0.24$_{a,x}$	0.01	0.013
d14 – 29	0.33$_{y}$	0.39$_{x,y}$	0.41$_{x}$	0.37$_{x,y}$	0.02	0.050
d0-29	0.23	0.24	0.24	0.24	0.02	0.948
Daily feed intake, kg/d						
d4 – 14	0.21$_{b,y}$	0.26$_{a}$	0.18$_{b}$	0.25$_{a,x}$	0.02	0. 002
d14 – 29	0.42$_{b,y}$	0.56$_{a,x}$	0.45$_{x,y}$	0.54$_{x}$	0.05	0.019
d0-29	0.28	0.36	0.29	0.34	0.03	0.050
Gain:Feed						
d4 – 14	0.90$_{b,y}$	0.93$_{a}$	1.23$_{b}$	0.94$_{a,x}$	0.07	0.004
d14 – 29	0.85	0.72	0.94	0.79	0.09	0.227
d0-29	0.88	0.69	0.82	0.69	0.13	0.279
No. treated[2]	21	33	27	16		
Total amount treated[2], %	22.58	37.50	34.18	17.98		

[1]Includes all pigs from blocks 1 and 2. Common diet provided: Phase 1, d0 - 4; Phase 2, d4 – 14; Phase 3, d14 – 29; Phase 4, d29 - d42.

[2]Value represents onsite animals as pigs housed in the southeast farm were not included in this value as none, excluding two who were castrated late, were treated during the course of the study.

Table 3.8 Distribution of pigs tested positive for fecal color change and at each weigh period into categories to d29 post-wean.

	Control	**Citristim**	**Mint Oil**	**γ-tocopherol**	**Chisq**
Fecal Color Change, %					
Day 2					
Positive	4.7	12.2	10.1	11.1	0.127
Negative	95.3	87.8	89.9	88.9	
Day 3					
Positive	31.8	35.3	32.4	39.2	0.513
Negative	68.2	64.7	67.6	60.8	
Day 4					
Positive	66.9	56.4	66.9	64.1	0.182
Negative	33.1	43.6	33.1	36.0	
Weight Category Distribution, %					
Day 4					
<5.1 kg	29.1	27.6	25.5	19.7	0.188
5.15 to 7.2 kg	50.0	44.8	55.0	51.3	
≥ 7.25 kg	21.0	27.6	19.5	28.9	
Day 14					
< 7.26 kg	34.5	27.9	20.0	22.2	0.114
7.27 to 9.9 kg	45.3	48.1	55.9	51.0	
≥ 9.95 kg	20.3	24.0	24.1	26.9	
Day 29					
< 12.6 kg	36.5	25.5	17.4	23.5	0.013
12.65 to 16.5 kg	41.9	46.4	56.3	53.0	
≥ 16.55 kg	21.6	28.1	26.4	23.5	

Table 3.9 Performance of grow-finish pigs up to d126 after weaning at the on-site facility.

Items	Control	Citristim	Mint Oil	γ-tocopherol	SEM	P-value
	Lightweight pigs					
Body weight, kg						
d42	16.1$_{c}$	17.8$_{bc}$	19.3$_{ba}$	20.5$_{a}$	0.86	<.0001
d70	34.1$_{x}$	36.1$_{x,y}$	38.8$_{y}$	38.6$_{z}$	1.72	0.034
d98	55.8$_{x}$	58.1$_{x,y}$	62.3$_{y}$	60.2$_{x,y}$	2.54	0.096
d126	85.0	84.2	92.6	87.6	3.71	0.178
Daily gain, kg/d						
d29 – 42	0.37$_{b}$	0.32$_{b,x}$	0.57$_{a}$	0.17$_{c,y}$	0.05	<.0001
d42 – 70	0.47$_{b}$	0.39$_{b,x}$	0.75$_{a}$	0.20$_{c,y}$	0.07	<.0001
d70-98	0.49$_{b,y}$	0.42$_{b}$	0.77$_{a,x}$	0.28$_{b}$	0.08	0.002
d98-126	0.67$_{b}$	0.52$_{bc}$	1.01$_{a}$	0.33$_{c}$	0.11	0.0001
	Heavyweight pigs					
Body weight, kg						
d42	26.0	28.2	27.3	26.1	1.10	0.132
d70	49.0	50.6	50.0	48.8	1.64	0.596
d98	73.4	74.2	74.8	72.9	2.46	0.870
d126	102.5	102.4	105.0	102.2	3.24	0.850
Daily gain, kg/d						
d29 – 42	0.35	0.67	0.56	0.62	0.21	0.109
d42 – 70	0.44	0.61	0.65	0.70	0.19	0.163
d70-98	0.43$_{y}$	0.69$_{x,y}$	0.68$_{x,y}$	0.81$_{x}$	0.23	0.078
d98-126	0.52$_{y}$	0.79$_{x,y}$	0.82$_{x}$	0.90$_{x,y}$	0.23	0.077

Table 3.10 Performance of grow-finish pigs up to d126 after weaning at the off-site facility.

Items	Control	Citristim	Mint Oil	γ-tocopherol	SEM	P-value
	Lightweight pigs					
Body weight, kg						
d42	$18.3_{x.y}$	15.1_{y}	19.1_{x}	18.8_{x}	1.6	0.027
d70	44.6	43.2	45.4	46.0	1.8	0.537
d98	74.4	70.0	71.4	74.6	2.3	0.238
d126	104.6	100.3	101.1	106.5	2.7	0.177
Daily gain, kg/d						
d29 – 42	0.14	0.12	0.09	0.18	0.04	0.220
d42 – 70	1.00	1.03	0.97	1.01	0.05	0.573
d70-98	1.04_{a}	0.94_{c}	0.91_{b}	1.02_{ac}	0.03	0.006
d98-126	1.03	1.04	1.02	1.02	0.06	0.979
	Heavyweight pigs					
Body weight, kg						
d42	28.8	28.4	29.3	26.2	1.84	0.532
d70	58.3	61.5	61.4	58.4	3.03	0.532
d98	87.2	89.9	90.5	89.3	3.92	0.807
d126	112.1	115.7	120.4	118.7	4.64	0.276
Daily gain, kg/d						
d29 – 42	0.26_{a}	0.12_{b}	0.18_{ab}	0.18_{ab}	0.03	0.038
d42 – 70	1.04_{x}	1.20_{y}	$1.16_{x,y}$	$1.17_{x,y}$	0.06	0.054
d70-98	0.92	0.98	1.01	1.07	0.05	0.128
d98-126	0.95	1.01	1.09	1.11	0.06	0.181

Table 3.11 Antioxidant content in serum, colostrum, and milk samples by treatment.

Items	Control	Citristim	Mint Oil	γ-tocopherol	SEM	P-value
GSH Colostrum and Milk, U/ml						
Colostrum	3.38	3.01	2.95	3.21	0.85	0.976
d4 Milk	7.16	7.43	7.38	7.61	1.72	0.998
d14 Milk	7.92	11.8	12.5	9.77	2.29	0.464
SOD Serum, U/ml						
Breed	9.94	9.83	9.79	9.72	1.34	0.999
d110 Gest.	18.5	18.7	13.5	16.6	2.75	0.360
Weaning	9.76	11.82	10.69	10.09	1.38	0.504

Table 3.12 Antioxidant content in serum, colostrum, and milk samples in gilts vs multiparous sows.

Items	Gilts	P1+	SEM	P-value
GSH Colostrum and Milk, U/ml				
Colostrum	4.39$_a$	2.75$_b$	0.69	0.045
d4 Milk	6.29	7.67	1.76	0.485
d14 Milk	11.3	10.2	2.29	0.704
SOD Serum, U/ml				
Breed	8.73	10.7	1.29	0.149
d110 Gest.	14.4	18.2	2.68	0.186
Weaning	9.60	11.4	1.27	0.166

CHAPTER 4

4.0 General Discussion

Assessment of the inclusion of different feed additives on the mitigation of oxidative stress during gestation and lactation on the sow and her offspring's performance was the focus of this thesis. It was hypothesized that the inclusion of Citristim, peppermint oil, and ɣ-tocopherol in gestation and/or lactation diets would reduce the state of oxidative stress in the sow during gestation and lactation and thus potentially improve her performance and the performance of her offspring in the suckling and post-weaning period. Genetic selection practices by commercial breeding companies has resulted in a more hyper prolific sow. Increased metabolic burden as a result of greater number of pigs born alive per litter, has ensued in these prolific females to fall under a systemic type of oxidative stress (Berchieri-Ronchi et al., 2011;Tan et al.,2015). Appearance of oxidative stress during gestation can impact fetal development, such as the occurrence of IUGR offspring as well as piglets born with birth defects that can hinder growth and ultimately lead to pre-weaning mortality. During lactation, oxidative stress arises as mobilization of body reserves to meet the demands of milk production to support offspring consumption for growth occurs. Impairment of milk production can hinder piglet growth during the suckling period, resulting in lighter pigs at weaning. In conjunction with the impact on offspring performance, a sow under a constant catabolic state can undergo drastic weight loss which effects overall production cost; Strathe et al. (2017) note excessive mobilization would be expensive as the body reserve loss must be reconstituted during the following gestation, resulting in increased feet cost.

Citristim, a *Pichia guilliermondii* byproduct from citric acid fermentation, has been reported to be an immunomodulator when included in poultry diets (Shanmugasundaram and Selvaraj, 2012; Shanmugasundaram et al., 2013); however, it was found to only work locally and not systemically in the body as the immune parameters measured in the spleen did not differ from the control group while parameters in the cecal tonsils were altered. Successfulness of prebiotic or probiotic yeast-based products in livestock diets has been observed to vary from case to case. Inclusion of Citristim at 0.1% of the diet in lactation resulted in no difference in colostrum and milk immunoglobin content between Citristim- and control-fed sows (Bass et al., 2019). However, alteration of other immune parameters (i.e. increased concentration of leukocytes and IL-1 with decreased IL-10 expression) were observed when Citristim was fed to poultry and swine alike (Shanmugasundaram and Selvaraj, 2012; Bass et al., 2019). In Chapter 2 and 3, ability of the additives to reduce oxidative status of the dam during gestation and lactation was ineffective as minimal difference was detected for SOD and GSH concentration compared to control. For yeast cell, this may be a result of insufficient antioxidant activity that is supplemented to consumers of the product. Farrugia and Balzan (2012) denote that yeast cells express limited pools of antioxidants which sufficiently protect the yeast itself against ROS. Ability of Citristim to bind pathogens found in the intestines may in part help the animal's immune system as less toxins passing the basolateral membrane of the small intestine leading to illness (Pulske et al., 2018). Reducing illness may allow the body to utilize nutrients for mitigating the effects of elevated ROS concentration as opposed to both fighting off a sickness and attempting to lessen the detrimental effects caused by high ROS levels.

Phytochemicals are components generated in plants. A single plant has been found to contain vast numbers of classes of phytochemicals all which offer support to the plant. For centuries, medicinal and homeopathic use of phytochemical oils, often coined as essential oils, has occurred. Although medicinal use of plants and their bioactive compounds is not a new concept, there has been an upsurge in research evaluating their use in the livestock and food industry as a means to prevent rancidity and also as alternatives to in feed antibiotics. There is an increasing number of publications that investigate the use of phytochemicals in inhibiting the overproduction of ROS in lab and livestock animals (Bourque et al., 2012; Tan et al., 2015; Zou et al., 2016). The peppermint oil used in both Chapter 2 and 3 possesses various antioxidant components that potentially could reduce the oxidative stress that a sow undergoes during the gestation and lactation period (Wu et al., 2019a). It was reported that the most abundant biochemical components in peppermint were menthol (39.3%) and menthone (25.2%; Bassolé et al., 2010). Interestingly, both menthol and menthone are considered to be polyphenols. Oh et al. (2013) note that phenolic compounds are widely known to act as free radical scavengers by their reducing properties. Thus, it was hypothesized that the inclusion of peppermint oil in these studies would lower oxidative status of the dam. However, as observed in both Chapter 2 and 3, females fed diets supplemented with peppermint oil in fact required a larger concentration of SOD to reduce circulating superoxide ions in the body at d110 of gestation. Increased levels of the superoxide ion in the body may indicate that the animal is under a state of oxidative stress as Konzack and Kietzmann (2014) report that expression of SOD molecules correlates with ROS levels explaining the need for larger amounts of the enzyme. Total GSH in colostrum for both

studies indicated that there was a lower GSH concentration circulating in milk. A lower concentration may signify that the female is under a state of stress as GSH is utilized to activate GPX for detoxification of hydrogen peroxide into water. As the concentration of GSH in milk was 0.50 to a 1 U/ml lower in peppermint oil-fed females, it in agreement with SOD results indicating that the peppermint oil group was under a state of oxidative stress. However, despite being in an elevated state of oxidative stress, piglet performance, BW and weight category distribution, in the suckling and post-wean period improved. It has yet to be determined why offspring from peppermint fed sows had improved performance in the suckling and post-wean period. As noted by Lushchak (2012), there are varying types of oxidative stress that a cell undergoes. Considering that females from the peppermint oil group were in a more elevated state of stress, the system can become "quasistationary" or is stabilized at a new, higher level of ROS. A higher level of stabilized ROS in both the sow and her offspring may impact the degree of stress that must occur before negative consequences to the body system occurs.

In Chapter 2, one of the four diets contained a combination of both CitriStim and peppermint oil. The combination of feed additives was included as a dietary treatment because it was believed that the presence of the two and their differing chemical components would lead to a synergistic effect. Peppermint oil contains various bioactive compounds, more importantly antioxidants that can assist in reducing the concentration of ROS present in cells. Whereas Citristim offers support as an immunomodulator and has the ability to bind to pathogens located in the small intestine. However, it was observed that females and offspring provided this diet did not perform better than the groups given the test diets containing the ingredients individually. The SOD and GSH

concentrations in serum and milk also did not differ from that of the control group. A potential explanation for the lack of synergistic effects may due to other bioactive compounds present in peppermint. Işcan et al. (2002) determined that peppermint oil possessed a weak antimicrobial activity. Although antimicrobial activity of peppermint oil is weak its ability to inhibit microorganism is still present and, with Citristim binding pathogens, the two may have worked similarly resulting in performance to be comparable to groups fed additives separately. Based on this lack of synergistic effect peppermint oil and Citristim were evaluated separately in the study reported in Chapter 3.

The main objective of the two studies reported herein were to evaluate the effectiveness of various feed additives included in pregnant and lactating sow diets on reducing oxidative stress in the dam. It was believed that by reducing sow oxidative status, performance of both sow and her offspring would improve. However, neither oxidative status nor sow performance differed among treatment groups. Lack of impact by inclusion of feed additives on sow performance was consistent, with lack of impact on sow antioxidant status being inconsistent, with several other studies (Tan et al., 2015; Meng et al., 2018; Bass et al., 2019). It also was hypothesized that offspring performance would improve with the inclusion of the different feed additives in maternal diets. Interestingly, difference in offspring BW and weight category distribution in the suckling and post-wean period where exposure to peppermint oil resulted in heavier pigs was observed in both studies. It was noted that light weight offspring from peppermint oil-fed sows were heavier in the post-wean period up to market. Intriguingly, a pattern was observed in both studies that when provided diets containing peppermint oil after weaning, or exposure to peppermint oil via maternal diet supplementation, a larger

percentage of pigs deemed lightweight were characterized as average weight in subsequent weigh periods than pigs with exposure to control diets.

As a means to further assess the implications of the test ingredients on maternal immune functions and the transmission to offspring, in Chapter 3, immunocrit in both colostrum and piglet serum at d2 of age was analyzed. Piglet sera immunocrit across the three weight categories and colostral immunocrit was not altered by maternal dietary treatment. Vallet et al. (2013) developed this method of analyzing sera immunocrit as a means to inexpensively and rapidly measure sera Ig. Similar to Bass et al. (2019), sows provided diets containing Citristim did not differ in colostral IgG compared to control. It appears that the inclusion of the additives, especially Citristim, did not alter immune parameters. Vallet et al. (2013) state that the concentration of sera IgG dilutes as the piglet grows. As well, the concentration of immunoglobins absorbed decreases as the gut "closes up" as the young animal ages (Blum and Baumrucker, 2008). Thus, the age of piglets when samples were collected may have impacted the ability to detect differences. It was reported that the concentration of serum IgG and immunocrit in piglets at d2 is relatively lower than at d0 (Vallet et al., 2013). Thus, samples collected at day 2 may not indicate total amount of IgG consumed by offspring indicating that immunocrit should be collected at day 1 may better reflect differences in piglet robustness at birth or sow colostrum supply. In both studies, litters with more than 15 pigs were split suckled at least twice a day in the first 48h after birth, this management tool may also have contributed to the lack of difference in serum immunocrit among weight categories.

IGF-1 concentration in both milk and sow sera was analyzed to provide insight into the impact on piglet performance. IGF-1 is thought to be involved in growth and

development of young animals (Laron, 2001). Serum concentration of the growth hormone in offspring is utilized as an indicator of nutritional status (Richards et al., 1991; 1995; Bossis et al., 1999). Most notably, inadequate consumption of colostrum by piglets can inhibit growth as secretion of IGF-1 by the liver in piglets is minimized. As oxidative stress has a negative impact on milk production, circulating IGF-1 in offspring could potentially decrease as the animal is not consuming adequate nutrients. It has been reported that caloric restriction is associated with a reduction in growth hormone (GH) receptor mRNA transcriptions (Maes et al., 1988; Straus and Takemoto, 1990). As IGF-1 production is stimulated by GH, alteration of receptors and in turn reduction in GH signaling could hinder secretion of IGF-1 leading to delayed growth. Reducing dam oxidative status with the inclusion of additives that can aid in maintaining the balance between maternal antioxidant and ROS production, could potentially ensure piglet growth is not hindered. In concert with sufficient nutrient supply, differences in IGF-1 transfer to offspring via milk may influence offspring performance. It is believed that the source of colostrum and milk IGF-1 could be traced back to maternal serum, as the mRNA expression from mammary glands is relatively low. Discovery of an abundance of receptors for IGF-1 in the intestinal lining of neonatal and newly born animals (Schober et al., 1990) suggest that offspring can utilize both endogenous (i.e. dietary IGF-1) and exogenous source of IGF-1. Xu et al. (2002) found that IGF-1 in milk is likely to survive the proximal region of the small intestine, supporting the use of colostral or milk IGF-1 concentration as an indicator of pig growth potential. The concentration of the IGF-1 in sow sera was less in comparison to levels in colostrum and milk, supporting the presumption that the source of colostral and milk IGF-1 in maternal serum. However, the

lack of difference in sow sera, colostrum, and milk IGF-1 suggests some other factor is involved in the improvement of piglet performance observed in both studies.

In conclusion, although dietary inclusion of Citristim, peppermint oil, and ɣ-tocopherol was not as effective in reducing the oxidative status of the dam as expected, offspring performance, particularly opportunity pigs, improved. The inclusion of phytochemical oils in sow diets, particularly peppermint oil, would be advantageous based on the responses reported herein. Results observed in both studies were similar, thus inclusion of peppermint oil only in lactation diets may be sufficient to achieve the desired benefit to offspring performance. Addition of peppermint oil in gestation or lactation diets would be suitable in improving offspring performance throughout the suckling and post-weaning periods.

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